

SOME STUDIES ON SYNERGISM OF CADMIUM AND pH
WITH REFERENCE TO CERTAIN ASPECTS OF METABOLISM
OF THE CRAB *OZIOTELPHUSA SENEX SENEX* FABRICIUS
(ARTHROPODA : CRUSTACEA)

Thesis submitted to Sri Venkateswara University
for the award of degree of
DOCTOR OF PHILOSOPHY

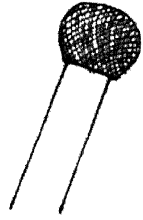
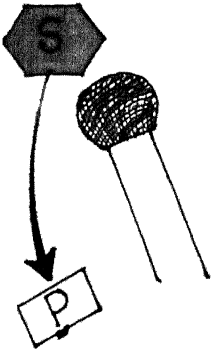
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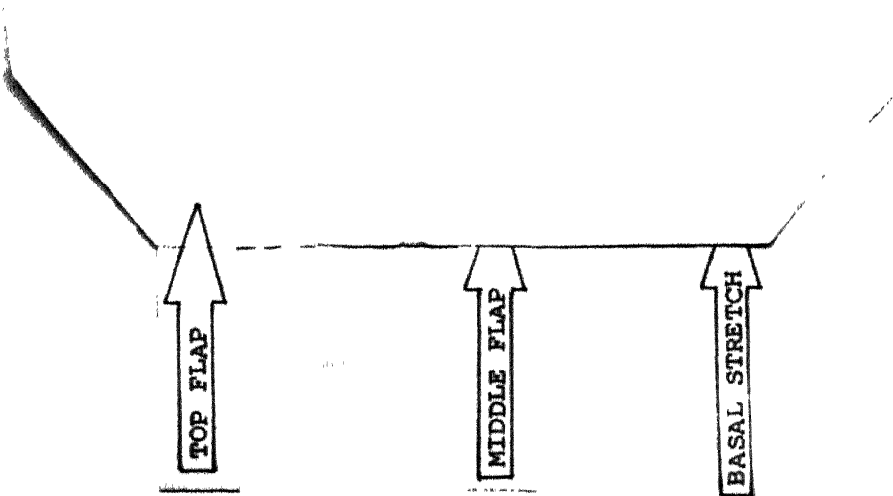
Research Guide

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SISOM TRADUC PATHWAYS



DEDICATED
TO
MANAM'S FAMILY

PREFACE

Increasing rate of industrialization going on in developing countries has no doubt the sunny aspect of economic uplift of the masses but the dark, villainous aspect of it also should be taken note of. Cadmium ecotoxication and acid rain that go hand-in-hand with the progress of the due of civilization — industrialization processes, provide clearcut illustrations of the villainy mentioned above.

The industrial giant Japan has had to bear the burnt of the industriogenous diseases like the 'Itai-Itai' syndrome. This is the human connection of industriogenous pathology of a society. The terrific-tentacles of this pathology extend into several areas of biological activity like various aquatic ecosystems. Once again, this effect may be fed back into the 'hominid' biology.

Every nation, on the path of industrial progress, will have to face this hazard exemplified in the 'Japanese connection' noted above. India is not an exception.

It is this prospect for India that was agitating the mind of the author for a long time. And precisely this agitation provided the motive to the author to embark on an investigative programme, to contribute useful data-lines in the field of cadmium and acid

toxicology. The author has identified certain investigative inadequacies in this area of toxicology (Chapter I) and has planned and executed inquisitorial programme accordingly (Chapters II to VII).

The chapteral locations may contain an overdose of theorination ('theoreorrhoea'; if we may call it so) and verbogenesis (creation of new terms). These may be viewed against the exuberant enthusiasm of the author to tap to the maximal extent the potential of datal import.

The work is by no means exhaustive and many investigative lacunae may be visible to the critical eye.

The author submits that he will come forward with further more searching insights into the areas of toxicology exposed here inadequately, due to lack of temporal and physical facilities.

The reader is asked this question: Is there at least one insight that appeals to your 'critical' 'approbatory' think-self?

If the answer is the 'yeal' positive then.... great satisfaction will become the author's preserve. From which, he gets inspiration to make many more investigative strides.

ACKNOWLEDGEMENTS

Virtually nothing is equal to the unwincing mind, constant cooperation, careful observation and valuable suggestions of my guide and mentor, Dr. Prof. Chandrasekharam Vedam, Professor & Head of the Department of Zoology, Dr. Sri Manavala Ramanujam Research Centre in Biology, S.C.S. Arts College, Tirupati, in completion of this dissertation in the present shape. He has been a source of great inspiration and confidence in research pursuits right from my under-graduate level. His fatherly affection which enliven my spirits is never forgettable. It is really a great gift and privilege for me to work under his enthusiastic guidance.

I am grateful to Prof. V. Rangaswami, former Principal, who took pains in establishing this Research Centre, and Major B. Papa Rao, present Principal for their constant encouragement. I am thankful to the management of T.T. Devasthanams for providing facilities.

I express my sincere thanks to Professors K.S. Swami, R. Ramamurthi, Department of Zoology, School of Biological & Earth Sciences, S.V. University, Tirupati for their help and encouragement.

I am indebted to my teachers Drs. P. Satyam, V. Doraswami Reddy, N. Sreeramulu, T. Haragopal and

Sri K. Balarami Reddy and Sri A. Nagaraja Rao, Librarian for their ceaseless cooperation and encouragement. I also thank my laboratory assistants for their help during my work and other teaching and non-teaching staff of the college, for their encouragement.

I express my heartfelt thanks to Dr. N.S. Srikanth for his assistance during laboratory experimentation. I also thank my research colleagues from University, Drs. P.S. Reddy, K.S. Jagannatha Rao, G. Harold Philip and Sri P.R. Aravinda Babu for their innate cooperation and help.

I am specially thankful to my beloved friends -- Paddu, Chitti, Dr. Prasad, Murali, AVSes, Phaniraj, Jayaram, Sampath, Suresh, Sankar, Dr. Ranganath, Dr. Raghava Rao, Raja Reddy, Vasu, Sethu, Ravi, Madhu, Sekhar, Raja, Geeni, GR, Siva, Rajee, Ramu, Siva Prasad, Raghupathy, Damodaram, Paul, Bhaskar, Raghu and Kodandaram for their time to time enquiries and encouragement.

I am highly indebted to my father, Dr. Radha Mohan Rao; my mother, Smt. Ranga Devi; my sister, Dr. Usha Polisetty and my brother-in-law, Dr. Rayudu Polisetty (Kentucky, USA) whose moral support and encouragement helped me a lot in finalising this work.

My special thanks are due to Messers V.S. Rajan, B. Ravi Kumar, Nagoor, P.S. Ramakrishnudu and Seshu

for their technical support which made the presentation of this thesis possible in this form.

I am grateful to University Grants Commission and Council of Scientific and Industrial Research, New Delhi for their financial help.

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CHAPTER I

GENERAL INTRODUCTION

CHAPTER I

GENERAL

INTRODUCTION

Cadmium pollution and acid rain are amongst the most serious environmental hazards extending their villainous influences into every area of biosphere.

I 1 LITERATURE CITATIONS

I 1.A CADMIUM POLLUTION

Cadmium-cause 'Itai-Itai' syndrome noted in Yokohama Prefecture, Japan some years ago (Friberg et al.,

1974) may be taken as a standard for the harm this metallic toxicant perpetrates to the human and infra-human biosystems.

Several reports highlight the polluting presence of cadmium in the various ecosystems (Kobayashi et al., 1975; Salomons and Forstner, 1980; Baudó et al., 1981; Muller, 1981; Muntau, 1981; Houba and Remacle, 1982; Taylor, 1982; De Bernardi et al., 1983) and the intoxicating presence in biosystems (Bonnell, 1955; Mount and Stephen, 1967; Uthe and Bligh, 1971; Lewis et al., 1972; Havre et al., 1973; Friberg et al., 1974; Hutcheson, 1974; Shuman et al., 1974; Van Hook, 1974; Elinder et al., 1976; Gornes and Muntau, 1976; Martin et al., 1976; Zarogian and Cheer, 1976; Calamari and Marchetti, 1977; George and Coombs, 1977; Nimmo et al., 1977; Pentreath, 1977; Thurborg et al., 1977; Ostergaard, 1978; Ash and Lee, 1980; Ray et al., 1980b).

The toxic influence of cadmium is well documented (Severi, 1896; Prodan, 1932; Wilson et al., 1941; Schroeder et al., 1965; Lewis et al., 1969; Gardner and Yevich, 1970; Gilluly, 1970; Nilsson, 1970; Stowe et al., 1972; Itokawa et al., 1973; Larsson, 1975; Johansson and Larsson, 1978).

The physiological and biochemical influence of cadmium on various facets of biological systems is also well studied (Richman, 1958; Piscator, 1962; 1966, 1978; Piscator and Axelsson, 1970; Hiltibrau, 1971; Bukima, 1972; Nordberg and Piscator, 1972; Sangalang and Freeman, 1974; Wald et al., 1974; Nomiyama, 1975, 1978, 1979; Koyama and Itazawa, 1977; Nechay and Saunders, 1977, 1978; Nogawa et al., 1977; Piavaux, 1977; Vernberg et al., 1977; Bingham et al., 1978; Duke et al., 1978; Sakurai, 1978; Bernard et al., 1979; Moraitou-Apostolopoulou and Verriopoulos, 1979; Taniguchi et al., 1979; Tucker, 1979; Bonner et al., 1980; Pecon and Powell, 1981; Tohyama et al., 1981).

I 1.B ACID RAIN TOXICOLOGY	Various aspects of acid rain ecology and toxicology have been studied by numerous investigators (Dahl, 1927; Ellis, 1937; Jones, 1948; Harrison, 1958; Bishai, 1960; Parsons, 1968; Calabrese, 1969; Beamish and Harvey, 1972; Sutchliffe and Carrick, 1973; Almer <u>et al.</u> , 1974; Grahm <u>et al.</u> , 1974; Hendrey and wright, 1975, 1976; Schofield, 1975; Borgstorm and Hendrey, 1976; Borgstorm <u>et al.</u> , 1976; Dovland <u>et al.</u> , 1976; Hendrey <u>et al.</u> , 1976; Likens, 1976; Likens <u>et al.</u> , 1977; Savita Samant and Agarwal, 1977; Snekvik, 1977;
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Chintawar, 1978; Karuppasamy, 1979; Raddum, 1979, 1980; Fryer, 1980; Hoback and Raddum, 1980; Miller and Mackay, 1980; Okland, 1980a,b,c; Jan Okland and Okland, 1980; Parent and Cheetham, 1980; Murthy et al., 1981a,b; Ramalingam and Raghunathan, 1981; Maiti, 1982; Walton et al., 1982; Mastanamma, 1984; Burman, 1985).

I 1.c STUDIES ABOUT INDIAN SITUATION

The extensive review by Nath (1986) on Cadmium toxicity with special reference to Indian conditions shows that considerable contribution is made with respect to distribution of cadmium in Indian eco-systems. Much of the work pertains to vertebrates involving development of several vertebrate models including primate model. The focus of attention of this work is on human health vis-a-vis cadmium intoxication. Work with invertebrates has been only scanty.

I 1.D. IDENTIFICATION OF INVESTIGATIVE LACUNAE

The literature citations reveal the clear investigative inadequacy for the Indian ecosystems and biological systems, both with reference to cadmium toxicology and acid-base toxicology. Further, interaction between

these two factors have also not been looked into critically, but for a few reports: For example, Haines (1981) has reported an ecosystem-pH interaction with regard to retention of cadmium.

I 2 PROPOSITION OF THE PROBLEM

Keeping the inadequacies mentioned above, the investigative programme of the present dissertation was chalked out. The investi-

gative programme included the individual (in severo) and combinational (in combinatio) influence of Cd and acidic pH on the respiratory metabolism and tissue organic composition and metabolism in a selected freshwater animal.

Thus, this work may serve as a contribution to the list of Indian works in the area of toxicology of the factors cadmium and acidity and may also provide insights into the interaction between these two factors.

I 3 THE ANIMAL

The local freshwater rice field crab, Oziotelphusa senex senex has been selected as the experimental

organism. The organism is an important invertebrate inhabitant of rice-field ecosystem. It forms an

important food item to agricultural communities in certain parts of South India. Study of its physiology under cadmial and other heavy metal stress gains importance at places where the animal habitats are not at safe distances from industrial toxic effluvia. The various aspects of . physiology and biochemistry, with regard to different stresses like temperature (Ramamurthi, 1967; Ramamurthi, 1967); salinity (Venkata Reddy, 1976) and pesticide stress (Bhagyalakshmi, 1981; Sreenivasula Reddy et al., 1983) have been amply worked out in this organism. The seasonal biochemical cycles (Raghupathy, 1983) and induced ecdysial cycles (Reddy, 1982) have been worked out.

With the accrual of such investigative databases in different areas of stress-response biology, this organism became a natural choice for use in the present investigative programme.

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CHAPTER II

TOXICITY EVALUATION

As a prelude to systematic investigation on the individual and combined effects in vivo of cadmium and pH on the physiology and biochemistry of the local fresh water field crab Oziotelphusa senex senex, toxicity parameters were determined for these two kinds of pollutants. The data are presented here (Tables II.1 and II.2; Fig. 2.1). From the data the following lethality (toxicity) parameters and sublethal (experimental) concentrations were derived at:

LC ₅₀ 48h for Cd	6.0 ppm
SLC for Cd	0.6 ppm
LpH ₅₀ for 48 h	4.5
SLpH	..,	..	6.0

TABLE II.1: Effect of Cd on the mortality rate of Oziotelphusa senex senex after 48h exposure

Concentration of Cd (ppm)	*Percent kill	**Probit kill
3	0	0
4	8.3	3.59
5	25.0	4.33
6	50.0	5.00
7	76.0	5.67
8	91.7	6.34
9	100.0	8.09

*Percent kill calculated as the number of animals found dead at the end of the exposure period (48 h) % number of animals exposed to the stressant medium.

**Probit kill computed according to the procedure of Finney (1964).

The values (% kill, probit kill) are averages of 6 determinations for each concentration of the stressant medium. Cd for preparation of the stressant media used as CdCl_2 .

Animals used: Laboratory-adapted crabs, fed on frog leg/earthworm diet and maintained in glass aquaria. Each experiment was started with 12 animals exposed to the stressant medium.

Characteristics of water used in Cd-versus mortality studies:

Temperature: $27^\circ \pm 2^\circ\text{C}$

pH: 7.1 - 7.4

Hardness: 61 mg/l (as bicarbonates)

Dissolved oxygen: 5.38 ± 0.76 ml/l

TABLE II.2: Effect of pH on the mortality rate of Oziotelphusa senex senex after 48h exposure

pH value of the medium	% Mortality*
5.5	8.6 \pm 0.95
5.0	25.0 \pm 6.3
4.5	50.0 \pm 8.5
4.0	83.0 \pm 4.3
3.5	92.0 \pm 2.7
3.0	100.0 \pm 10.8

*Each value represents mean \pm S.D of 6 determinations.

Number of animals used: 10 per determination.

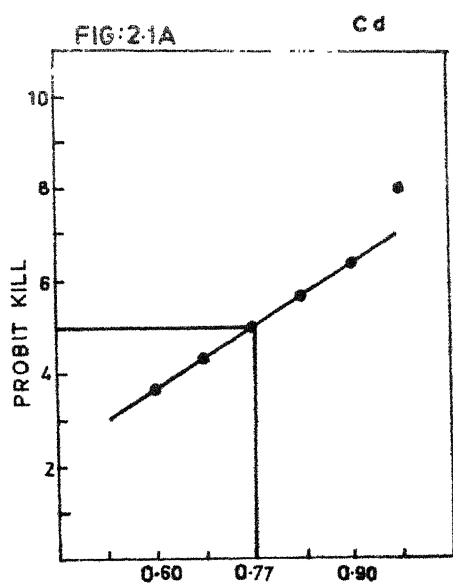
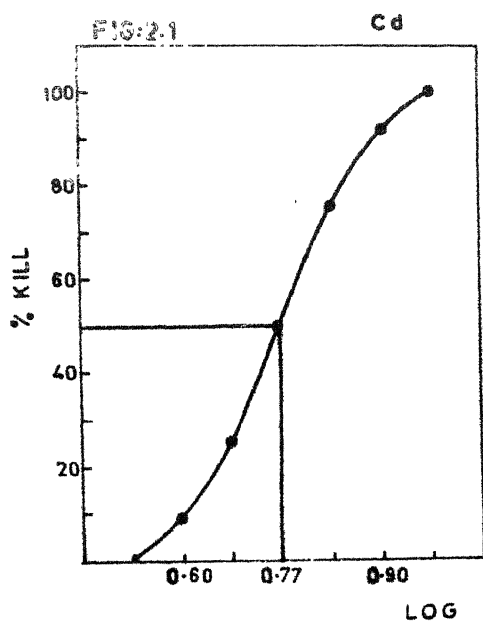
Design of experiment: according to Anderson (1944)

For preparation of pH media HCl: H₂O buffer was employed (Oser, 1979).

Characteristics of water used in pH-versus mortality studies: as indicated under table II.1 except pH.

Almost similar LC₅₀ and probit kill values are noted for a local snail Pila globosa under Cu and Hg stresses (Balavenkatasubbaiah, 1984) and in the fish, Tilapia mossambica under cadmium stress (Usharani, 1986, Unpublished data).

FIGs. 2.1 & 2.1A: The effect of Cd on acute mortality of O. senex senex. $LC_{50/48h}$ occurred at log concentration of 0.77 corresponding to 6 ppm of Cd concentration.



CHAPTER III

OXYGEN CONSUMPTION

III 1 INTRODUCTION

The measurement of oxygen consumption is often used as an experimental investigative procedure to assess

the influence of various metabolism-influencing factors and agents on the physiology and metabolism of an organism. The present chapter deals with the profile of oxygen consumption in the crab, O. senex senex vis-a-vis the physiochemical factors viz., pH and cadmium in individual and combinational regimes.

III 2 MATERIALS AND METHODS

Oxygen consumption was estimated in the animals of the control and experimental groups (please vide Chapter II for the experimental regimes used in the present work).

For estimation of aquatic oxygen consumption of the animal the method of Winkler (in Welsh and Smith, 1953) as modified by Saroja (1959) was adopted using a wide-mouthed bottle of 600 ml capacity as respiratory chamber (Fig. 3.1A). The temperature of the ambient of the respiratory chamber was maintained within the narrow limits of 27°-31°C to minimize the temperature effects.

LEGEND FOR FIGURE 3

FIG 3.1A: Set up of apparatus used for measuring the whole animal oxygen consumption.

- A - Reservoir
- B - Inlet tube from the reservoir
- C - Respiratory chamber
- D - Thermometer
- E - Air inlet tube
- F - Outlet tube from the Respiratory chamber
- G - Sample collecting bottle
- H - Rubber stopper

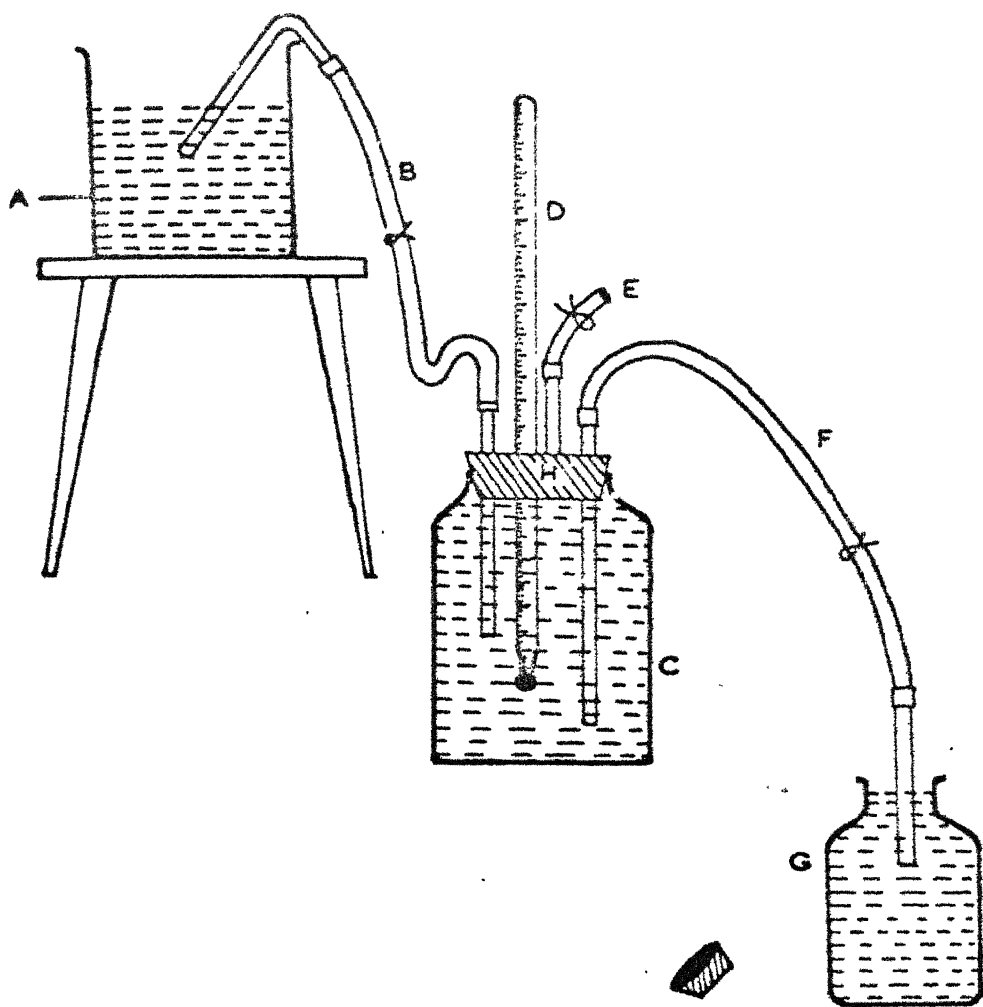


FIG 3.1A

The procedural steps concerning collection of experimental biological material viz., crabs (O. senex senex), their in-laboratory maintenance and preparation of these organisms for experimental investigations have been described in several publications that emerged from this laboratory and others (Venkata Reddy, 1976; Raghavaiah, 1977; Raghavaiah et al., 1980; Reddy, 1981; Bhagyalakshmi, 1981 and Raghupathy, 1984).

Preparation of the different experimental groups was essentially according to the guide-points enumerated earlier (Vide Chapter II).

In each of the stressant media organisms were maintained for use in long-term (30 days) stressant-influence studies. The medium was changed daily and the animals were fed outside the stressant medium and replaced into the medium. Feeding was effected with ad libitum rations of earthworms and frog legs.

The data were evaluated to obtain insights into the effects of the stressants using standard statistical analytical techniques (Pillai and Sinha, 1968).

III 3 RESULTS

The stressants, Cd and pH, influence the metabolic profile of the crab, variably, as a function of time^{31a and Fig 3.1} course of exposure (Tables 3.1). Both the stressants cause, an immediate increase in the rate

of oxygen consumption. The elevation of oxygen consumption by pH (+ 274%) is greater than that caused by Cd (+ 199%), two-days post-stress. As contrasted, with this remarkably steep elevation of oxygen consumption, the de-elevation of this rate immediately afterwards i.e., four days post-stress is equally cataclysmic. The fluctuations or 'spasms' of metabolism at subsequence time-intervals post-stress, present a picture of apparent disorder of metabolism. Of the two stressants, Cd factor appears to exert a more constant and orderly influence on the respiratory rate of the organism. The alteration of respiratory rate of the organism is consistently in the 'elevatory phase' during the earlier periods of stress and the depressive phase of the subsequent periods of exposure to the stressant is almost equally consistent. The 'phase change' in metabolism-alteration caused by the stressant appears to occur between the 12th and 14th day post-stress and to put in more empirical terms, 2 weeks post-stress.

The pH factor too appears to exhibit a phase shift metabolism-alteration, but with less dramatic conspicuity.

The interaction of the factors with regard to the metabolism-alteration in the crab or more explicitly the 'combinational' influence of the factors, Cd and pH

on the metabolism is perceptible only in the earliest period of exposure i.e., two days post-stress. For this period, the metabolism-alteration is in the elevation-direction no doubt, but this elevation (+ 55.0%) is 'dwarfed' when compared with the elevations caused by Cd (+199.0%) and pH (+274.0%) factors. Besides, the phase shift mentioned above in metabolism-alteration appears to be advanced to shorter post-stress period under combinational regime of the stressants.

III 4 COMMENT

The immediate increase in the oxygen consumption rate and its depression during subsequent periods of exposure to the stressants are in agreement with the literature reports (Heistand, 1931, 1940; Raymont and Sheild, 1964; O'Hara, 1972; Thurberg et al., 1979 and Dean Kettle et al., 1980). However, the exceptionally steep increased noted under Cd (+199.00) and pH (+274.00) stress are in contrast to the reports (Elston, 1983) that increases in oxygen consumption beyond 150% are toxic to organisms in general. This initial 'shoot-up' of oxygen consumption is of the category of overshoot reaction and is of a shorter duration (i.e. one day). How this ephemeral overshoot is tolerated by the organism is immediately inexplicable.

Under the stressant regimes, a general trend of depression of metabolism is noted. Under the combi-national regime, oxygen consumption is depressed remarkably as compared to the effects of individual Cd and pH regimes (on the 14th day).

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TABLE 3.1: Effect of individual and combined in vivo stress of Cd & pH on Unit metabolism of O. senex senex.
(Values, expressed as ml/g/h, are mean \pm S.D of 5 determinations).

Day post-stress	Control	Cd			pH			Combined (Comb)		
		Unit metabolism	Change % Control	Unit metabolism	Unit metabolism	Change % Control	Unit metabolism	Unit metabolism	Change % Control	
2 nd	0.0291 ± 0.0185	0.0870 ± 0.0064	+ 199.00	0.1088 ± 0.0190	+ 274.00	0.0451 ± 0.0090	+ 55.00			
4 th	0.0821 ± 0.0250	0.1025 ± 0.0016	+ 24.85	0.0718 ± 0.0130	- 12.55	0.1151 ± 0.0085	+ 40.20			
6 th	0.0777 ± 0.0086	0.0950 ± 0.0060	+ 22.30	0.0930 ± 0.0080	+ 19.70	0.0712 ± 0.0063	- 8.40			
8 th	0.0626 ± 0.0230	0.0680 ± 0.0052	+ 8.63	0.0812 ± 0.0250	+ 29.71	0.0744 ± 0.0090	+ 18.85			
10 th	0.0512 ± 0.0066	0.0910 ± 0.0106	+ 77.73	0.0446 ± 0.0166	- 12.90	0.0353 ± 0.0043	- 31.10			
12 th	0.0571 ± 0.0083	0.0694 ± 0.0053	+ 21.54	0.1320 ± 0.0200	+ 131.20	0.0508 ± 0.0083	- 11.10			
14 th	0.1005 ± 0.0084	0.0830 ± 0.0107	- 17.40	0.0566 ± 0.0046	- 43.70	0.0523 ± 0.0043	- 48.00			
16 th	0.1004 ± 0.0175	0.0955 ± 0.0160	- 4.90	0.1264 ± 0.0250	+ 25.90	0.0487 ± 0.0110	- 51.50			
18 th	0.0853 ± 0.0150	0.0602 ± 0.0080	- 29.40	0.0749 ± 0.0151	- 12.20	0.0560 ± 0.0057	- 34.35			
20 th	0.0584 ± 0.0101	0.0604 ± 0.0110	+ 3.42	0.1041 ± 0.0150	+ 78.25	0.0460 ± 0.0092	- 21.20			
22 nd	0.0623 ± 0.0240	0.0520 ± 0.0077	- 16.50	0.0460 ± 0.0090	- 26.20	0.0680 ± 0.0130	+ 9.15			
24 th	0.0914 ± 0.0245	0.0380 ± 0.0125	- 58.40	0.0513 ± 0.0091	- 43.87	0.0522 ± 0.0090	- 42.90			
26 th	0.0806 ± 0.0062	0.0674 ± 0.0056	- 16.40	0.0547 ± 0.0056	- 32.10	0.0527 ± 0.0024	- 34.60			
28 th	0.0421 ± 0.0221	0.0580 ± 0.0121	+ 37.80	0.0568 ± 0.0085	+ 35.00	0.0550 ± 0.0150	+ 30.64			
30 th	0.0513 ± 0.0073	0.0880 ± 0.0066	+ 71.54	0.0580 ± 0.0175	+ 13.06	0.0640 ± 0.0095	+ 24.76			

TABLE 3.1a: Comparison of mean values of 'normal' Unit metabolism of O. senex senex with the mean values for animals subjected to individual and combined stress conditions presented in Table 3.1.

F = 152.44

CD = 0.00526

Day. Post-stress	Comparison with		
	Cd	pH	Comb
2nd	S	S	S
4th	S	S	S
6th	S	S	S
8th	S	S	S
10th	S	S	S
12th	S	S	S
14th	S	S	S
16th	NS	S	S
18th	S	S	S
20th	NS	S	S
22nd	S	S	S
24th	S	S	S
26th	S	S	S
28th	S	S	S
30th	S	S	S

S = Significant 5% level; NS: Not Significant
Critical difference (CD) value was calculated according to the formula

$$t_o = \frac{t_{\alpha} \sqrt{\sigma_e^2 (1/k_1 + 1/k_2)}}{\quad} \text{ where}$$

t_o = tabulated value of 't'

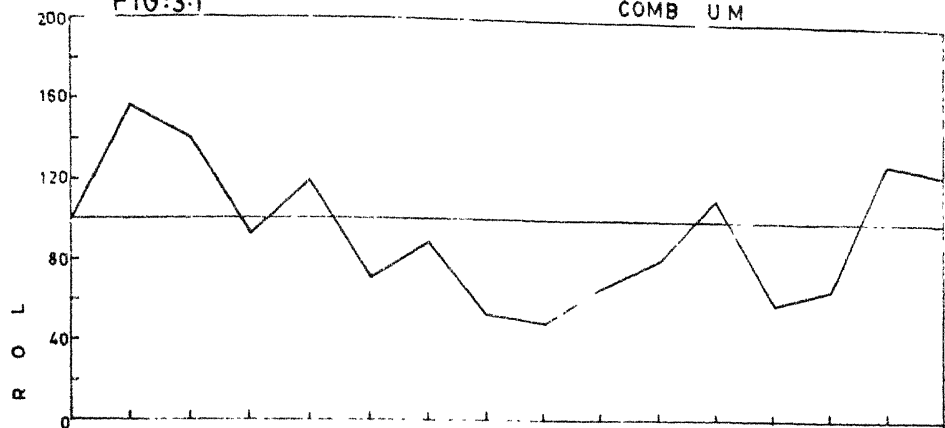
σ_e^2 = error mean square and

k_1 & k_2 = numbers of observations based on which two means to be compared.

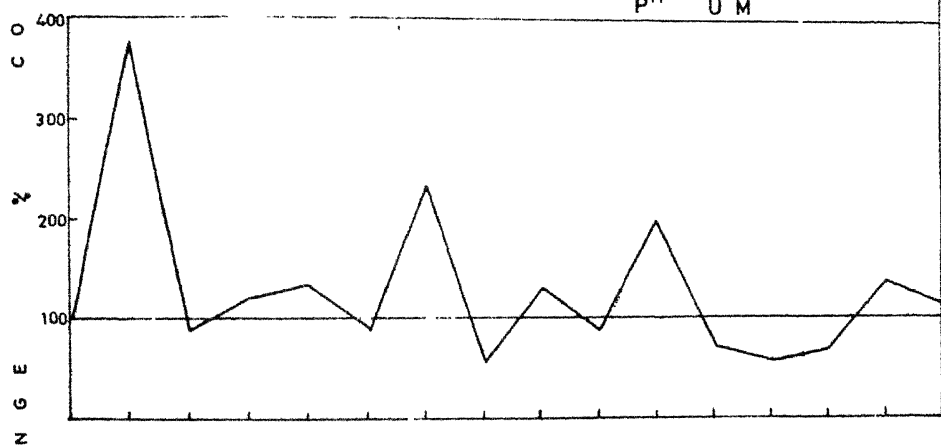
FIG. 3.1: Time course (in days) response of Unit metabolism (UM) of O. senex senex at sublethal Cd (0.6 ppm)-, pH (6.0)- and their combinational (Comb) concentratio: states.

FIG:3.1

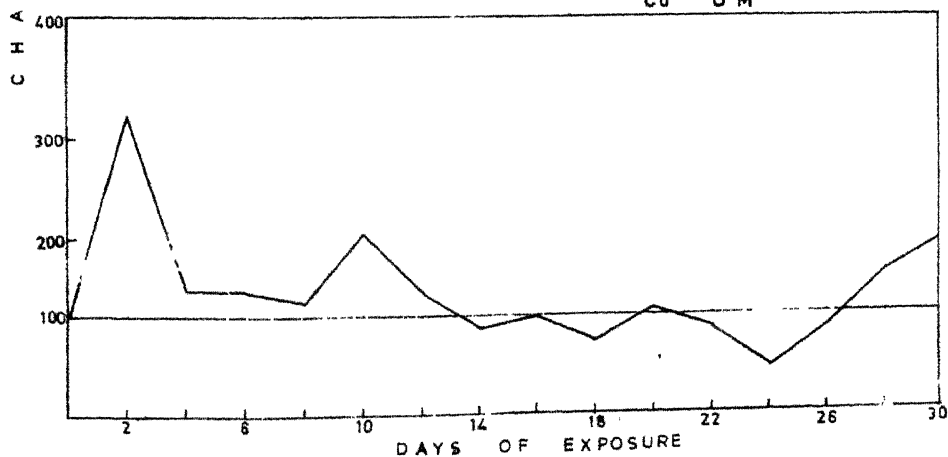
COMB U M



P^H U M



Cd U M



CHAPTER IV

ORGANIC COMPOSITION OF TISSUES

The rates and routes of organismic metabolism are affected by environmental toxicants and stressants. Consequently, the organic composition of the tissues of the organism is affected. Conversely, the deviation of organic composition of an organism under a stressant-regime reflects the toxic influence of the stressant on the metabolic milieu of the organism.

IV 1 INTRODUCTION

The present chapteral location is apportioned to an examination of the tissue organic composition of the fresh water field crab O. senex senex as affected by pH- and Cd- regimes, individually (in severo) and combinationally (in combinatio).

The stressant regimes used in the study are given elsewhere (Chapter II). The durations of stress selected for study are 15 and 30 days post-stress.

IV 2 MATERIAL AND METHODS

IV 2.A THE INVESTIGATIVE GROUPS

The crabs, collected from the fields around Tirupati, were maintained in laboratory aquaria, being fed ad libitum with froglegs, earthworms and cockroaches. The animals were given a laboratory adjustment duration of one week and later the experiment was commenced.

In separate aquaria, four batches of animals were maintained; (1) the untreated or unstressed controls; (2) Cd-stressed experimentals; (3) pH-stressed experimentals and (4) experimentals under combinational stress of Cd and pH. The details regarding the concentrations of stressants used are given elsewhere (Chapter II). The maintenance protocol for the different experimental batches are also given at an earlier location (*vide ut supra*).

Two periods of stress were chosen for the analysis of biochemical composition of the tissues of the crab: 15 days post-stress (15 dps) and 30 days post-stress (30 dps). These durations are chosen keeping in view the fact that the crab in question has been found to undergo almost complete adjustment to experimentally imposed stresses like salinity (Krishnamoorthy and Srihari, 1973) 30 days post-stress; with regard to toxins like the organophosphate pesticide malathion/sumithion, the metabolism-turning point has been identified to occur about 15th day post-stress in the organism (Bhagyalakshmi, 1981).

The animals (experimentals and controls) were sacrificed at the end of the stress-periods mentioned above and the tissues, viz., hepatopancreas (HP).

IV 2.B THE TISSUES

chelate leg muscle (M), gill (G) and cephalothoracic ganglionic mass (CTGM) according to operative procedures detailed by other workers (Raghavaiah, 1977; Raghavaiah et al., 1980). The haemolymph (HL) was collected using hypodermic syringe. The biochemical estimation procedures were carried out on wet chilled tissues, as soon after isolation as practicable.

The following biochemical

IV 2.C THE ESTIMATION constituents were estimated
 METHODOLOGY in the tissues mentioned
 above.

1. Estimation of total acid-precipitable and acid-extractable anthrone positive substances:

The estimations were carried out in the tissues using anthrone as the principal sugar-reacting agent, colorimetrically.

The tissues were homogenized in 5% (W/V) trichloroacetic acid (TCA) and after centrifugation (at 2500 rpm), the supernatant was used for estimation of total acid-extractable anthrone-positive substances (TAEAPS). In the TCA-residue, the total acid-precipitable anthrone positive substances (TAPAPS). For this purpose, the TCA residue was dissolved in 0.4M sodium hydroxide, and the resultant solution was used. The anthronometric estima-

tions were carried out according to Carroll et al., (1956). Colorimetry was carried out in Bausch and Lomb spectronic 20 Colorimeter.

2. Estimation of total protein content:

For estimation of total protein content (TP) of tissues, the TCA-precipitate is used. The NaOH-solution of TCA residue (please vide above) was used for the estimation of TP using Folin-Ciocalteu reagent colorimetrically (Lowry et al., 1951) in Bausch and Lomb Spectronic 20 Colorimeter.

3. Estimation of soluble protein (SP) content of tissues:

The organic fraction, SP-content, was estimated in sucrose (0.25 M) homogenization supernatants, according to the procedure laid down in Lowry et al., (vide ut supra).

4. Estimation of total ninhydrin-positive substances (TNPS):

The organic component, total ninhydrin-positive substances (TNPS) include free amino acids and estimation of TNPS content is a fairly acceptable method for determining the quantitative disposition of these 'organic osmotic effectors' in the tissues. For estima-

tion of TNPS-content of the tissue, the TCA-extract is used. The estimation-procedural details were according to Moore and Stein (1957, 1968).

5. Estimation of total lipids (TL) in tissues:

The TL-content of the tissue was estimated employing the method envisaged by Folch et al., (1957).

6. Estimation of haemolymphatic organic constituents:

Since the haemolymph is a fluid tissue, the homogenization procedures adopted for the solid tissues (vide ut supra) are not necessary in haemolymphatic chemical analyses.

The haemolymph, drawn with hypodermic syringe was used after centrifugation and eliminations of residue, for the estimation of extra-cellular protein (ECP), Folinometrically (i.e., using Folin-Ciocalteu phenol reagent), and estimation of total ninhydrin positive substances (extra-cellular). The TAPAPS, TAEAPS and TP estimations however, were carried out after treatment of the 'whole blood' with TCA, as in the case of solid tissues (vide ut supra). The estimation of total lipid also was carried out using 'whole blood'.

The stressants both in severo
and in combinatio exert consis-
tently a depressive influence on

IV 3 RESULTS

IV 3.A CEPHALOTHORACIC the TAEAPS (Table 4.1; Fig. 4.1)
GANGLIONIC MASS
(CTGM) content of the cephalothoracic
ganglionic mass (CTGM) of the
crab, O. senex senex. Statistical analysis shows that
the alterations in TAEAPS content caused by the stressants
as compared to the controls are significant (Table 4.1a)
15d-treatment of the organisms with the stressants is
associated with greater quantum of reduction of TAEAPS-
content of CTGM as compared to the 30-day-treatment.
For both stress or treatment durations, the combinational
treatment is associated with a higher quantum of depre-
ssion as compared to the individual treatments. In
combinatio, the stressants appear to 'interact', in as
much as the TAEAPS depression effect is higher (-50.0%)
under combinational regime than any of the individual
regimes. But one can not easily visualize the pheno-
menon of 'potentiation' in these effects going by the
definition of this term in toxicological or stressant-
biological literature. The situation is essentially
similar for the 30 day stress duration where the combi-
national effect on TAEAPS (-41.4%) is almost an
'additive' picture of the effects under the individual

stressant regimes (Cd: -17.24% + pH: +21.4% = - 38.64%) (Table 4.1).

The TAPAPS-pool of CTGM is elevated consistently by the stressants both in severo and in combinatio 15 days post-stress (15 dps) (Table 4.2; Fig. 4.2). Cd-stress causes greater TAPAPS-elevation (+ 57.6%) than pH-stress (+ 31.0%). In combination, the stressants cause TAPAPS-elevation (+82.0%) which is much higher than the individual effects.

At the longer stress-duration, i.e., 30 dps, a different effect picture is evident. Cd and pH, at this stress-duration are associated with diametrically opposite effects. Cd exerts a TAPAPS-elevatory effect (+ 41.0%) whereas pH exerts a TAPAPS-depressive effect of an almost equal magnitude (-45.5%). In combination, the stressants cause a depression (-45.5%) of the TAPAPS-content of CTGM (Table 4.2). The variations in CTGM TAPAPS-content caused by the stressants are found to be statistically significant (Table 4.2a).

The total protein (TP) content of CTGM is found to be elevated by both stressants, in severo and in combinatio, at both durations post-stress (Table 4.3; Fig. 4.3). At the shorter exposure-duration, the elevations of TP-content are greater than the elevations at

the longer exposure-duration. Fifteen days post-stress, the elevation caused by Cd (+ 166.0%) is greater than that caused by pH (+ 100.0%). In the combinational regime, the TP-augmentative effect (+ 133.4%) is higher than that caused under pH-regime but lower than that caused under Cd-regime.

At the longer exposure-duration (i.e., 30 dps), the combinational regime is associated with greater TP-pool elevatory effect (+ 86.1%) as compared to Cd-regime (+ 34.3%) or pH-regime (+ 72.8%) (Table 4.3). The variations in the size of TP-pool of CTGM caused by different treatments mentioned above are found to be statistically significant (Table 4.3a).

The soluble protein (SP) content of CTGM is notably influenced by the stressants, especially pH. Cd-stress, both at shorter and longer exposure-durations, causes a slight depression of SP-content (-10.8%, 15 dps; -8.4%, 30 dps). In contrast, pH-stress leads to perceptible elevation of SP-content, 15 dps (+ 35.0%) and more remarkable elevation for the longer exposure-duration (+ 92.0%; 30 dps) (Table 4.4; Fig. 4.4). Under the combinational regime, the elevatory effect at the shorter exposure-duration (+13.4%; 15 dps) is relatively feeble in comparison with the

effect obtained for the longer exposure-duration (+ 108.5%). The effects of SP-content of CTGM obtained for the different experimental stressant-treatments are found to be statistically significant (Table 4.4a).

The variations in the levels of total ninhydrin positive substances (TNPS) of CTGM caused by different treatments are given in table 4.5. The results of the experiment are graphically represented in fig. 4.5. The statistical treatment of the data of table 4.5 is appended in table 4.5a.

At the shorter exposure-duration (15 dps) both Cd and pH lead to the elevation of TNPS-content of CTGM. Cd causes a more notable elevation (+ 62.2%) than pH (+ 17.0%). These stressants, however, in combination cause a depressive effect of considerable order (-20.0%) At the longer exposure-duration, the stressants, both individually and combinationally cause depressions of TNPS-content of CTGM. In individual regimes, the effect of Cd-stressant (- 32.3%) is far greater than that caused by pH-stressant (- 4.0%). In the combinational regime, the depression (- 15.5%) is greater than that under pH-regime but smaller than that resulting under Cd-regime (Table 4.5).

The total lipid (TL) content of CTGM of O. senex senex is consistently depressed by the stressants, both in severo and in combinatio, both at the shorter and longer exposure-durations (Table 4.6; Fig. 4.6). The depressions noted at the shorter exposure-duration (Cd: -55.23%; pH: -51.57%; combinational: - 49.44%) are notably greater than those noted at the longer exposure-duration (30 dps; Cd: -26.1%; pH: -22.75%; combinational: -20.68%).

The variations in TL-content of CTGM are statistically significant (Table 4.6a).

IV 3.B MUSCLE (M)
(CHELATE LEG
MUSCLE)

The data on the TAEAPS-content of M of O. senex senex are given in table 4.7 in relation to shorter- and longer-term stress of Cd and pH. The data denote a consistent depressive influence of the stressants on this component of the 'organic complement' of M. Cd-stress causes greater depression of TAEAPS-pool after the shorter stress-duration (- 60.0%) than after longer stress-duration (41.4%; Table 4.7; Fig. 4.7). The acid (pH) stress, on the other hand causes smaller decrement of TAEAPS-pool after shorter stress-duration (-28.6%) than after longer stress-duration (-46.0%). The depressive influence of the combinational regime on TAEAPS-

pool is greater for both stress-durations as compared to the changes inflicted by the individual regimes of the stressants. In this regime, the shorter stress-duration is associated with a higher reduction of TAEAPS-pool size (- 76.5%) than the longer stress-duration (- 61.5%).

The data presented in table 4.7 are found to show statistically significant variation (Table 4.7a).

The TAPAPS-pool of M also is subjected to a general depression under the stressant-regimes (Table 4.8; Fig. 4.8). After shorter-duration exposure, Cd causes a depression (-20.0%) of TAPAPS-pool of M and after longer-duration exposure, an elevation (+ 12.0%).

pH-stress causes a small depression (-5.0%) after shorter stress-duration and a notable depression after longer stress-duration (- 65.0%).

The two stressants in combination cause almost equally noteworthy depression of TAPAPS-pool of M after both stress-durations (- 68.0%, 15 dps; -73.0%, 30 dps; Table 4.8).

The variations in the TAPAPS-pool size in M shown in table 4.8 caused by the stressants are found to be statistically significant (Table 4.8a).

Cd and pH cause a general depression of the total protein (TP) - content of M of O. senex senex (Table 4.9; Fig. 4.9). Fifteen days post-stress, Cd leads to a 28.6% depression of this pool and after the longer duration of stress the depression is less considerable (-8.6%). pH-stress causes an elevation of TP-content (+ 14.2%) after shorter stress-duration and a depression (-21.4%) after the longer duration of stress. The two stressants in combination cause depressions after both shorter (-43.0%) and longer (-31.5%) stress-durations.

Analysis of variance (ANOVA) of the data on TP-content of M presented in table 4.9 has revealed that the variations in TP-pool size caused by the stressant-regimes are statistically significant (Table 4.9a).

The fraction of soluble protein (SP) of the protein pool of the chelate leg muscle tissue of O. senex senex is subjected to a consistent incremental influence by the stressant regimes, individual and combinational, after both shorter and longer durations of stress (Table 4.10; Fig. 4.10). The variations caused by the stressants are found to be statistically significant (Table 4.10a).

Cd-Stress leads to almost equal elevations of SP-content of M in both stress-durations (+50.0%, 15 dps; +46.0%, 30 dps). pH-stress causes a higher elevation of

SP-content, 30 days post-stress (+89.0%) than after 15 days of stress (+ 65.3%). In the combinational regime, the elevation of SP-content noted after longer stress-duration (+71.0%) is slightly greater than the elevation (+62.4%) noted 15 days after stress.

The level of total ninhydrin-positive substances (TNPS) of M of O. senex senex is generally incrementally influenced by the stressants (Table 4.11; Fig. 4.11).

Cd-stress causes a 33.0% elevation of TNPS-pool of M, 15 days post-stress. For the longer-stress duration, the pool is slightly depressed (-6.3%) and this depression is found to be statistically non-significant.

The TNPS-pool size of M is non-significantly elevated (+3.0%) 15 days post-stress by pH-stress. At the longer stress-duration, the pool is significantly elevated (+33.0%).

In the combinational regime, the TNPS-pool shows a depression of notable and significant quantum (-48.8%) in the shorter stress-duration. The elevation noted in the longer stress-duration (+76.4%) is more remarkable.

The total lipid (TL) pool of M of O. senex senex is decrementally influenced by Cd and incrementally by pH. Combinational regime is marked by elevation of this organic component pool (Table 4.12; Fig. 4.12).

Cd-stress causes a higher depression (-34.7%) of TL-pool of M in the longer duration of stress than in the shorter duration of stress (-24.0%).

pH-stress, on the other hand causes remarkable elevations of TL-pool, the elevation in the shorter stress-duration being more considerable (+127.0%, 15 dps; +71.2%, 30 dps).

The stressants, in their combinational regime cause a noteworthy elevation of TL-content (+86.6%), 15 days post-stress. In the longer duration of stress, the TL-content shows a less considerable elevation (+7.8%, 30 dps).

The variation of TL-content of M under the stressant regimes is statistically significant (Table 4.12a).

IV 3.C HEPATOPANCREAS (HP) Hepatopancreatic TAEAPS-content of O. senex senex is generally decrementally influenced by the stressants under study (Table 4.13; Fig. 4.13).

Cd-stress is associated with a shorter-stress-duration decrement (-37.7%) of a smaller magnitude as compared with the decrement under longer-stress-duration (-64.3%).

pH-stress causes almost equal depressions of TAEAPS-content of hepatopancreas (HP) at both stress durations (-56.6%, 15 dps; 45.0%, 30 dps).

In the combinational regime, the shorter stress-duration leads to a notable depression of TAEAPS-level of HP (-50.0%) whereas in the longer stress-duration, a small and statistically non-significant elevation (+10.0%) is noted.

Statistical treatment of the data with regard to TAEAPS-content of HP (Table 4.13a) shows the quantitative variation caused by the stressants to be significant.

The TAPAPS-content of HP of O. senex senex is positively (i.e., incrementally) modified by the stressants, Cd and pH, in severo and negatively (i.e., decrementally) in the combinational regime (Table 4.14; Fig. 4.14). The variations caused by the stressants are statistically significant (Table 4.14a).

Cd, in its individual regime causes a notable increase of hepatopancreatic TAPAPS-level, 15 days post-stress (+56.0%) and the increase is still greater, 30 days post-stress (+83.0%). pH-stress causes a smaller increase of TAPAPS-level of HP (+36.3%) 15 days post-stress, as compared to Cd. In the longer stress-duration the increment is found to be much less (+11.0%) and statistically non-significant.

In the combinational regime, a TAPAPS-level reduction is noted both in shorter (-22.0%) and longer (-40.0%) stress-durations.

The total protein (TP)-content of hepatopancreas of O. senex senex is consistently incrementally modified under the different stressant regimes of both longer and shorter durations (Table 4.15, Fig. 4.15). Cd causes a 90% increase in TP-content, 15 dps and in the longer stress-duration, the increase stands at 50% control. pH-stress causes a 20% increase during shorter stress-duration. During the longer stress-duration the increase of TP-content is more notable (+ 72.6%).

In the combinational regime, as in the case of pH-regime, the increment of TP-content is progressive, i.e., the increase is smaller for the shorter stress-duration (+ 24.2%, 15 dps) and greater for the longer stress-duration (+ 42.5%, 30 dps).

The variations in TP-pool of HP caused by the different stress regimes are found to be statistically significant (Table 4.15a).

The soluble protein (SP)-content of hepatopancreas of O. senex senex is modified in the positive (incremental) direction, consistently under the different stress regimes and durations studied (Table 4.16; Fig. 4.16). These variations are found to be statistically significant (Table 4.16a).

Cadmium-stress causes almost equal increases of SP-content of HP, during shorter and longer stress-durations (+ 21.4%, 15 dps; + 20.0%, 30 dps).

pH-caused increases of SP-content of hepatopancreas are progressive, i.e., smaller for the shorter stress-duration (+ 18.4%) and greater for the longer stress-duration (+ 38.7%).

The pattern of changes of SP-content under the combinational regime is similar to that observed under pH-regime i.e., progressive (+ 20.0%, 15 dps; + 33.4%, 30 dps).

The level of total ninhydrin-positive substances (TNPS) of hepatopancreas of O. senex senex is subjected to a general incremental influence by the different stressant-regimes (Table 4.17; Fig. 4.17).

Under Cd-regime, for both stress-durations, increases of TNPS-content are recorded. These increments follow a 'conservative' pattern: the shorter stress-duration being associated with a greater increase (+ 22.0%, 15 dps) and the longer stress-duration, with a smaller increase (+ 7.0%, 30 dps). Incremental changes noted under pH-stress also follow the 'conservative' pattern: the shorter stress-duration is asso-

ciated with a remarkable increase (+ 189.0%) of TNPS-content and with the prolongation of stress, the TNPS-content tends to reach the control level (+ 30.0%, 30 dps).

Under the combinational stress, the changes of TNPS-content of HP follow the progressive pattern: the shorter stress-duration is associated with a negative (decremental) change (-10.8%, 15 dps) and in the longer stress-duration, a notable positive (incremental) change is noted (+60.2%, 30 dps).

The variations of TNPS-content of HP of O. senex senex are found to be statistically significant (Table 4.17a).

The hepatopancreatic total lipid (TL) content of O. senex senex is consistently incrementally modified under the different stress-regimes and deviations studied (Table 4.18; Fig. 4.18). The variations are found to be statistically significant (Table 4.18a).

Fifteen days post-stress, Cd-stressed hepatopancreas shows a 38.5% increase in TL content and in the longer exposure- duration the TL-content decreases from the 15 dps-level to near-control level (+ 7.2%

control, 30 dps). Under pH-stress also a similar pattern of TL-content alteration is noted. Fifteen days post-stress, the TL-content stands at + 60.01 per cent control and for 30 days post-stress, the level of TL is only +27.4 per cent control.

In the combinational regime also, an initial higher percentage of increase of TL-content (+38.7% control, 15 dps) is followed by a return to normality during the longer stress-duration (+4.0% control, 30 dps).

IV 3.D GILL (G)

The levels of TAEAPS of gill tissue of O. senex senex are presented in table 4.19 and figure 4.19. A consistent

negative (decremental) influence is exerted by the stressant regimes on this constituent of the organic pool of gill of the crab.

The variations in TAEAPS-content of G noted under the different regimes of the stressants are found to be statistically significant (Table 4.19a).

Under Cd-stress, the TAEAPS-content of gill undergoes a decrease of notable quantum (-68.6% control, 15 dps) and after the longer exposure-duration the decrement of TAEAPS-content becomes less considerable

(- 31.4% control, 30 dps). Under pH-stress, the TAEAPS content undergoes almost similar quanta of decrease in both stress-durations (- 44.0% control, 15 dps; -41.0% control, 30 dps).

Under combinational regime, a 60.0% decrease of TAEAPS-content during the shorter exposure-duration is followed by a 68.0% decrease after the longer duration.

The data presented in table 4.20 (Fig. 4.20) show the influence of Cd and pH on the TAPAPS-content of G of O. senex senex. The stressants in severo cause initial increase and later (longer duration) decreases of TAPAPS-content while the combinational regime causes only decreases during both stress durations.

The variations in TAPAPS-content of G under the different stress-regimes are found to be statistically significant (Table 4.20a).

Under the Cd regime, a small increase of branchial TAPAPS-content (+ 9.0% control, 15 dps) and this is followed by a decrease, which is also small (- 15.6% control, 30 dps).

pH-regime on the other hand exert greater incremental influence in the shorter stress-duration

(+ 34.4% control, 15 dps) and decremental influence in the longer stress-duration (- 44.8% control, 30 dps).

In the combinational regime the TAPAPS-content of gill shows decrement in both shorter and longer stress-durations (- 31.8% control, 15 dps; - 46.0% control 30 dps).

Under the different stressant regimes studied, the branchial total protein (TP) content of O. senex senex undergoes a general incremental change (Table 4.21 Fig. 4.21).

The variations of TP-content of gill under the regimes are found to be statistically significant (Table 4.21a).

Under Cd-stress, the branchial TP-pool shows an enhancement of 31.0%, 15 dps. For the longer stress-duration, the enhancement is found to be only 10% control.

pH-stress causes a very slight negative change in branchial TP-content, initially (- 3.4% control, 15 dps). For the longer stress-duration, a considerable quantum of elevation (+ 46.4% control, 30 dps) has been recorded.

In the combinational regime, the shorter exposure-duration shows a 28.5% elevation of TP-content of G while for the longer exposure-duration, a more notable enhancement has been recorded (+ 71.3% control, 30 dps).

The branchial soluble protein (SP) content of O. senex senex is variably modified by the different stressant regimes (Table 4.22; Fig. 4.22), and these modifications are found to be statistically significant (Table 4.22a).

Cd-stress causes a remarkable increase (+ 71.2% control) of the SP-content of gill in the shorter exposure-duration. In the longer duration, a decrease has been noted (- 19.8% control, 30 dps).

pH-stress on the other hand causes a very small initial decrease of branchial SP-content (- 1.6% control, 15 dps) and a 40% increase in the longer stress-duration.

In the combinational regime, the change in the shorter stress-regime is considerable while change in the longer regime is very small (- 1.6% control, 30 dps) and statistically non-significant.

Tables 4.23 (Fig. 4.23) and 4.23a present the data on the levels of total ninhydrin-positive sub-

stances (TNPS) in the gill of O. senex senex, as a function of Cd and pH stress (in severo and in combinatio).

The TNPS-content of G, is consistently incrementally modified by the stressant regimes investigated.

Cd-stress causes an initial higher increase of TNPS-level of G (+ 21.1% control, 15 dps) and a smaller increment in the longer stress-duration (+ 10.0% control, 30 dps).

Fifteen days post stress, the branchial TNPS-level is elevated to 60.0% control and in the longer stress-duration the increase is very small and negligible (+ 2.3% control, 30 dps).

The combinational stress leads to a small elevation of gill TNPS in the shorter duration of stress (+ 5.1% control, 15 dps) and a larger elevation in the longer duration of stress (+ 26.0% control, 30 dps).

The branchial pool of total lipid (TL) in O. senex senex is variably modified by the stressant-regimes (Table 4.24; Fig. 4.24). The individual regimes of the stressants cause decremental changes consistently and the combinational regime causes incremental changes. These variations are statistically significant (Table 4.24a).

Cd-stressant caused decrements are smaller than pH-stressant caused decrements. Cd-stress causes a 26.3% decrement of TL-content of gill, in the shorter stress-duration. In the longer duration, the decrease is less considerable (-9.6% control, 30 dps).

pH-stress, in contrast, causes more profound decrements of branchial TL-content (-73.7% control, 15 dps; -45.1% control, 30 dps).

The combinational regime of the stressants causes a notable elevation of SP-content initially (+44.5% control, 15 dps). At end of the longer exposure-duration, the TL-content remains near control (+2.7% control, 30 dps)

The extra-cellular protein
IV 3.E HAEMOLYMPH (HL) (ECP) content of haemolymph
of O. senex senex undergoes
a general decremental change
under the different stressant regimes (Table 4.25; Fig. 4.25). The variations are found to be statistically significant (Table 4.25a).

Under the influence of Cd, a shorter duration depression of ECP of HL is caused (-23.7% control, 15 dps). During the longer stress-duration the haemolymphatic ECP-content is elevated (+28.0% control, 30 dps).

Under the influence of pH, depressions are caused both in shorter and longer stress-durations (-28.5% control, 15 dps; -11.8% control, 30 dps).

Under combinational regime also, depressions of HL-ECP are recorded in both stress-durations (-31.8% control, 15 dps; -69.1%, 30 dps).

The data given in table 4.26 (Fig. 4.26) show the changes of haemolymphatic TAEAPS-content in O. senex senex in the different stressant-regimes. A general depressive effect is evident in these regimes. These variations are statistically significant (Table 4.26a).

The TAEAPS-content of haemolymph undergoes decrements under both stress durations with Cd (-52.6% control, 15 dps; -31.0%, 30 dps).

Under pH-stress also, decrements of TAEAPS of haemolymph are recorded (-37.8% control, 15 dps; -25.0% control, 30 dps).

In the combinational regime however, the shorter stress-duration is marked by an increase (+24.0% control, 15 dps). In the longer stress-duration, a notable decrement is evident (-46.0% control, 30 dps).

The level of TAPAPS of haemolymph of O. senex senex is variably modified under the different stressant

regimes. (Table 4.27; Fig. 4.27). These variations are found to be statistically significant (Table 4.27a).

Under Cd-regime, the HL-TAPAPS is decrementally modified in the shorter stress-duration (-12.7% control, 15 dps). This change is statistically non-significant (Table 4.27a). In the longer stress-duration a small but statistically significant elevation is recorded (+25.6% control, 30 dps).

Under pH-regime, both stress-durations are associated with statistically non-significant alterations of TAPAPS of HL (-6.9% control, 15 dps; +7.2% control, 30 dps).

In the combinational regimes, notable, statistically significant depressions are evident in both stress-durations (-33.2% control, 15 dps; -47.9% control, 30 dps).

The haemolympathic total protein (TP) content is variably influenced by the different regimes. The shorter stress-durations are marked by depressions, consistently, and the longer durations, by elevations generally (Table 4.28; Fig. 4.28). These variations are found to be statistically significant (Table 4.28a).

The shorter duration Cd-stress is marked by a small statistically non-significant depression of HL-TP

(-13.8% control, 15 dps, and the longer duration, by an elevation (+23.8% control, 30 dps) which is statistically significant.

pH-stress causes only statistically non-significant changes of HL-TP in both stress durations (-10.8% control, 15 dps; +7.1% control, 30 dps).

Under combinational regime, the shorter duration decreament (-5.2% control, 15 dps) is not significant while the longer durations decrement (-22.5% control, 30 dps) is significant.

The haemolymphatic TNPS-content is consistently decrementally modified under the different stress-regimes (Table 4.29; Fig. 4.29). These variations are statistically significant (Table 4.29a).

Under Cd-regime, notable decrements of HL-TNPS are recorded in both shorter and longer stress-durations (-48.2% control, 15 dps; -65.7% control, 30 dps).

Under pH-regime, the longer stress-duration decrement (-51.4% control, 30 dps) is considerably higher than the shorter stress-duration decrement (-24.8% control, 15 dps).

Under the combinational regime, the shorter stress-duration is associated with a decrement (-28.3%

control, 15 dps) which is statistically significant and the longer duration, with a very small, statistically non-significant decrement (~ 3.0% control, 30 dps).

The haemolympathic total lipid (TL) of O. senex senex shows consistent incremental changes in the levels under all the different stress regimes and durations studied (Table 4.30; Fig. 4.30). These variations are found to be statistically significant (Table 4.30a).

Under both individual and combinational stresses, the incremental changes have been found to be consistently progressive, with reference to the stress duration, i.e., the longer stress-duration causing higher increment of HL-TL than the shorter stress-duration (Cd: + 84.40% control, 15 dps; + 150.0% control, 30 dps; pH : 75.0% control, 15 dps; + 172.0% control, 30 dps; combined stress: + 147% control, 15 dps; + 231% control, 30 dps).

The general depression alterations noted in the organic

IV 4 COMMENT

composition of the different tissues of O. senex senex, under different stressant regimes accord well with the literature reports on

stressant and toxicophysiology and biochemistry
(Mc Carthy, 1969; De Zwaan et al., 1975; Kazlaus-
kene and Schcherbina, 1975; Bubel, 1976; Mayer, 1977;
Rajarami Reddy, 1979; Gardner et al., 1981; Pecon and
Powell, 1981 and Balavenkatasubbaiah, 1984).

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TABLE 4.1: Effect of individual and combined in vivo stress of Cd & pH on TAEAPS of CTGM in O. senx senx.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 21.0 \pm 1.20

Stress	15 d	Change % Control	30 d	Change % Control
Cd	12.0 \pm 0.60	- 42.7	24.6 \pm 3.00	+ 17.2
pH	13.0 \pm 0.93	- 38.0	16.5 \pm 3.10	- 21.4
Combined (Comb.)	10.5 \pm 0.32	- 50.0	12.3 \pm 0.93	- 41.4

TABLE 4.1a: Comparison of means of TAEAPS of CTGM in
O. senex senex with reference to stress
conditions presented in Table 4.1.

F = 548.0

CD = 2.13

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	S	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 4.2: Effect of individual and combined in vivo stress of Cd & pH on TAPAPS of CTGM in O. senx senex.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 11.0 \pm 0.90

Stress	15 d	Change % Control	30 d	Change % Control
Cd	17.4 \pm 1.24	+ 57.6	15.5 \pm 1.55	+ 41.0
pH	14.4 \pm 0.74	+ 31.0	6.0 \pm 1.00	- 45.5
Combined (Comb)	20.0 \pm 1.00	+ 82.0	6.0 \pm 0.50	- 45.5

TABLE 4.2a: Comparison of means of TAPAPS of CTGM in O. senex senex with reference to stress conditions presented in Table 4.2.

F = 1241.72

CD = 1.22

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.3: Effect of individual and combined in vivo stress of Cd & pH on TP content of CTGM in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 150 \pm 20

Stress	15 d	Change % Control	30 d	Change % Control
Cd	400 \pm 55	+ 166	202 \pm 28	+ 34.6
pH	300 \pm 56	+ 100	260 \pm 27	+ 73.3
Combined (Comb)	351 \pm 40	+ 134	280 \pm 44	+ 86.6

TABLE 4.3a: Comparison of means of TP content of CTGM in O. senex senex with reference to stress conditions presented in Table 4.3.

F = 434

CD = 42.63

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.4: Effect of individual and combined in vivo stress of Cd & pH on SP content of CTGM in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 120.0 \pm 10.3

Stress	15 d	Change % Control	30 d	Change % Control
Cd	107 \pm 3.24	- 10.8	110 \pm 9.54	- 8.4
pH	162 \pm 3.40	+ 35.0	230 \pm 3.55	+ 92
Combined (Comb)	150 \pm 2.13	+ 13.4	250 \pm 22.0	+ 108

TABLE 4.4a: Comparison of means of SP content of CTGM in O. senex senex with reference to stress conditions presented in Table 4.4.

F = 1957.17

CD = 11.80

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	NS	S	S
Cd 15	-	S	S	NS	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.5: Effect of individual and combined in vivo stress of Cd & pH on TNPS of CTGM in O. senex senex.

(Values, expressed as μ grams of tyrosine/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 451 ± 16.3

Stress	15 d	Change % Control	30 d	Change % Control
Cd	732 ± 24.0	+ 62.2	305 ± 7.6	- 32.3
pH	527 ± 37.6	+ 17.0	433 ± 5.1	- 4.0
Combined (Comb)	358 ± 5.8	- 20.0	381 ± 6.4	- 15.5

TABLE 4.5a: Comparison of means of TNPS of CTGM in O. senex senex with reference to stress conditions presented in Table 4.5.

F = 4585.06

CD = 21.70

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	NS	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.6: Effect of individual and combined in vivo stress of Cd & pH on TL of CTGM in O. senex senex.

(Values, expressed as mg/g wet weight, are mean \pm S.D. of 6 determinations),

Control : 68.8 + 1.10

Stress	15 d	Change % Control	30 d	Change % Control
Cd	30.8 \pm 1.33	- 55.2	50.9 \pm 1.55	- 26.1
pH	33.3 \pm 1.72	- 51.5	53.2 \pm 1.35	- 22.7
Combined (Comb)	34.8 \pm 1.38	- 49.4	54.6 \pm 1.37	- 20.6

TABLE 4.6a: Comparison of means of TL of CTGM in O. senex senex with reference to stress conditions presented in Table 4.6.

F = 587.068

CD = 6.17

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	NS
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.7: Effect of individual and combined in vivo stress of Cd & pH on TAEAPS of M in O. senex senex.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 26.6 ± 2.60

Stress	15 d	Change % Control	30 d	Change % Control
Cd	10.4 ± 1.02	- 60.0	15.6 ± 1.30	- 41.4
pH	19.0 ± 1.55	- 28.6	14.3 ± 1.35	- 46.0
Combined (Comb)	6.25 ± 1.21	- 76.5	10.2 ± 1.33	- 61.5

TABLE 4.7a: Comparison of means of TAEAPS of M in O. senex senex with reference to stress conditions presented in Table 4.7.

F = 739.60

CD = 1.81

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.8: Effect of individual and combined in vivo stress of Cd & pH on TAPAPS of M in O. senex senex.

(Values, expressed as mg glucose/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 20.0 \pm 1.50

Stress	15 d	Change % Control	30 d	Change % Control
Cd	16.0 \pm 1.53	- 20.0	22.4 \pm 2.54	+ 12.0
pH	19.0 \pm 1.30	- 5.00	7.00 \pm 0.60	- 65.0
Combined (Comb)	6.40 \pm 0.75	- 68.0	5.40 \pm 0.60	- 73.0

TABLE 4.8a: Comparison of means of TAPAPS of M in O
senex senex with reference to stress condi-
tions presented in Table 4.8.

F = 835.0

CD = 1.65

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 4.9: Effect of individual and combined in vivo stress of Cd & pH on TP content of M in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 350 \pm 11.8

Stress	15 d	Change % Control	30 d	Change % Control
Cd	250 \pm 15.6	- 28.6	320 \pm 22.2	- 8.6
pH	400 \pm 24.1	+ 14.2	275 \pm 30.0	- 21.4
Combined (Comb)	200 \pm 16.2	- 43.0	240 \pm 14.5	- 31.5

TABLE 4.9a: Comparison of means of TP content of M in O. senex senex with reference to stress conditions presented in Table 4.9.

F = 1552.82

CD = 23.46

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.10: Effect of individual and combined in vivo stress of Cd & pH on SP content of M in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations).

Control: 114 \pm 16.0

Stress	15 d	Change % Control	30 d	Change % Control
Cd	170 \pm 27.6	- 50.0	166 \pm 23.6	+ 46.0
pH	188 \pm 26.0	+ 65.3	215 \pm 23.0	+ 89.0
Combined (Comb)	185 \pm 17.3	+ 62.4	195 \pm 23.7	+ 71.0

TABLE 4.10a: Comparison of means of SP content of M in O. senex senex with reference to stress conditions presented in Table 4.10.

F = 431.4

CD = 26.70

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	NS	S	NS
pH 15	-	-	NS	NS	S	NS
Comb 15	-	-	-	NS	S	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.11: Effect of individual and combined in vivo stress of Cd & pH on TNPS of M in O. senex senex.

(Values, expressed as μ grams of tyrosine/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 320 \pm 22.0

Stress	15 d	Change % Control	30 d	Change % Control
Cd	425 \pm 28.5	- 33.0	300 \pm 18.4	- 6.30
pH	330 \pm 23.1	+ 3.04	426 \pm 17.3	+ 33.0
Combined (Comb)	164 \pm 29.3	- 48.8	565 \pm 30.1	+ 76.4

TABLE 4.11a: Comparison of means of TNPS of M in O. senex senex with reference to stress conditions presented in Table 4.11.

F = 1673.0

CD = 28.81

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	S	S
Cd 15	-	S	S	S	NS	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.12: Effect of individual and combined in vivo stress of Cd & pH on TL of M in O. senex senex

(Values, expressed as mg/g wet weight, are mean \pm S.D. of 6 determinations).

Control: 37.3 + 1.09

Stress	15 d	Change % Control	30 d	Change % Control
Cd	28.4 \pm 1.13	- 24.0	24.4 \pm 1.18	- 34.7
pH	84.8 \pm 1.32	+ 127	63.9 \pm 1.40	+ 71.1
Combined (Comb)	69.7 \pm 1.22	+ 86.6	40.3 \pm 1.18	+ 7.80

TABLE 4.12a: Comparison of means of TL of M in Q.
senex senex with reference to stress
c.onditions presented in Table 4.12.

F = 13719.268

CD = 1.43

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 4.13: Effect of individual and combined in vivo stress of Cd & pH on TAEAPS of HP in O. senex senex.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 29.1 \pm 1.30

Stress	15 d	Change % Control	30 d	Change % Control
Cd	18.1 \pm 1.44	- 37.7	10.4 \pm 1.15	- 64.3
pH	12.6 \pm 1.86	- 56.6	16.0 \pm 4.44	- 45.0
Combined (Comb)	14.4 \pm 1.61	- 50.0	32.0 \pm 8.20	+ 10.0

TABLE 4.13a: Comparison of means of TAEAPS of HP in O. senex senex with reference to stress conditions presented in Table 4.13.

F = 210.07

CD = 4.39

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	NS
Cd 15	-	S	NS	S	NS	S
pH 15	-	-	NS	NS	S	S
Comb 15	-	-	-	NS	NS	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.14: Effect of individual and combined in vivo stress of Cd & pH on TAPAPS of HP in O. senex senex.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 10.0 ± 2.12

Stress	15 d	Change % Control	30 d	Change % Control
Cd	15.6 ± 4.00	+ 56.0	18.3 ± 5.31	+ 83.0
pH	13.6 ± 3.20	+ 36.3	11.1 ± 3.00	+ 11.0
Combined (Comb)	7.80 ± 0.88	- 22.0	6.00 ± 0.62	- 40.0

TABLE 4.14a: Comparison of means of TAPAPS of HP in O. senex senex with reference to stress conditions presented in Table 4.14.

F = 114.86

CD = 3.56

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	NS	S
Cd 15	-	NS	S	NS	S	S
pH 15	-	-	S	S	NS	S
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.15: Effect of individual and combined in vivo stress of Cd & pH on TP content of HP in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 221 \pm 21.0

Stress	15 d	Change % Control	30 d	Change % Control
Cd	420 \pm 16.7	+ 90.0	330 \pm 22.6	+ 50.0
pH	261 \pm 14.2	+ 20.0	382 \pm 13.6	+ 72.6
Combined (Comb)	275 \pm 12.2	+ 24.2	315 \pm 21.2	+ 42.5

TABLE 4.15a: Comparison of means of TP content of HP
in O. senex senex with reference to
stress conditions presented in Table 4.15.

F = 2288.6

CD = 20.84

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	NS
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 4.16: Effect of individual and combined in vivo stress of Cd & pH on SP content of HP in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 180 \pm 11.7

Stress	15 d	Change % Control	30 d	Change % Control
Cd	218 \pm 16.7	+ 21.4	216 \pm 17.8	+ 20.0
pH	214 \pm 29.0	+ 18.4	250 \pm 27.0	+ 38.7
Combined (Comb)	216 \pm 19.6	+ 20.0	240 \pm 13.2	+ 33.4

TABLE 4.16a: Comparison of means of SP content of HP in O. senex senex with reference to stress conditions presented in Table 4.16.

F = 837.17

CD = 23.64

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	NS	S	NS
pH 15	-	-	NS	NS	S	S
Comb 15	-	-	-	NS	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD Value was calculated according to the formula given in Table 3.1a.

TABLE 4.17: Effect of individual and combined in vivo stress of Cd & pH on TNPS of HP in O. senex senex.

(Values, expressed as μ grams of tyrosine/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 235 \pm 20.0

Stress	15 d	Change % Control	30 d	Change % Control
Cd	287 \pm 20.0	+ 22.0	251 \pm 26.2	+ 7.00
pH	680 \pm 20.0	+ 189	304 \pm 17.3	+ 30.0
Combined (Comb)	210 \pm 15.5	- 10.8	377 \pm 15.2	+ 60.0

TABLE 4.17a: Comparison of means of TNPS of HP in O. senex senex with reference to stress conditions presented in Table 4.17.

F = 2505.86

CD = 22.71

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	NS	S	S
Cd 15	-	S	S	S	NS	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.18: Effect of individual and combined in vivo stress of Cd & pH on TL of HP in O. senex senex.

(Values, expressed as mg/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 112 ± 1.81

Stress	15 d	Change % Control	30 d	Change % Control
Cd	156 ± 1.14	+ 38.6	120 ± 0.840	+ 7.15
pH	180 ± 1.06	- 60.0	143 ± 0.820	+ 27.3
Combined (Comb)	156 ± 1.01	+ 38.6	117 ± 3.54	+ 4.00

TABLE 4.18a: Comparison of means of TL of HP in O. senex senex with reference to stress conditions presented in Table 4.18.

F = 48492.02

CD = 2.01

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.19: Effect of individual and combined in vivo stress of Cd & pH on TAEAPS of G in 0. senex senex,

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 25.5 ± 2.00

Stress	15 d	Change % Control	30 d	Change % Control
Cd	8.00 ± 0.60	- 68.6	17.5 ± 2.66	- 31.4
pH	14.3 ± 1.06	- 44.0	15.0 ± 2.10	- 41.0
Combined (Comb)	10.0 ± 0.50	- 60.0	8.20 ± 0.66	- 68.0

TABLE 4.19a: Comparison of means of TAEAPS of G in O. senex senex with reference to stress conditions presented in Table 4.19.

F = 652.5

CD = 1.85

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	S	NS	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.20: Effect of individual and combined in vivo stress of Cd & pH on TAPAPS of G in O. senex senex.

(Values, expressed as mg of glucose/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 15.4 ± 0.80

Stress	15 d	Change % Control	30 d	Change % Control
Cd	16.7 ± 2.00	+ 9.00	13.0 ± 1.00	- 15.6
pH	20.7 ± 1.75	+ 34.4	8.50 ± 0.63	- 44.8
Combined (Comb)	10.5 ± 1.05	- 31.8	8.30 ± 0.80	- 46.0

TABLE 4.20a: Comparison of means of TAPAPS of G in O. senex senex with reference to stress conditions presented in Table 4.20.

F = 888.63

CD = 1.45

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.21: Effect of individual and combined in vivo stress of Cd & pH on TP content of G in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 191 \pm 15.5

Stress	15 d	Change % Control	30 d	Change % Control
Cd	250 \pm 10.6	+ 31.0	210 \pm 17.0	+ 10.0
pH	185 \pm 20.7	- 3.4	280 \pm 18.7	+ 46.4
Combined (Comb)	246 \pm 20.5	+ 28.5	328 \pm 13.7	+ 71.3

TABLE 4.21a: Comparison of means of TP content of G in O. senex senex with reference to stress conditions presented in Table 4.21.

F = 1465.55

CD = 19.95

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	S	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.22: Effect of individual and combined in vivo stress of Cd & pH on SP content of G in O. senex senex.

(Values, expressed as mg protein/g wet weight, are mean \pm S.D. of 6 determinations)

Control : 153 \pm 19.2

Stress	15 d	Change % Control	30 d	Change % Control
Cd	171 \pm 6.0	+ 11.8	123 \pm 18.1	- 19.8
pH	151 \pm 6.0	- 1.60	215 \pm 17.0	+ 40.5
Combined (Comb)	198 \pm 16.1	+ 29.0	150 \pm 9.53	- 1.60

TABLE 4.22a: Comparison of means of SP content of G in O. senex senex with reference to stress conditions presented in Table 4.22.

F = 990.5

CD = 16.61

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	S	S	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	NS
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.23: Effect of individual and combined in vivo stress of Cd & pH on TNPS of G in O. senex senex.

(Values, expressed as μ grams of tyrosine/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 300 ± 25.2

Stress	15 d	Change % Control	30 d	Change % Control
Cd	363 ± 25.7	+ 21.1	328 ± 20.0	+ 10.0
pH	480 ± 21.3	+ 60.0	307 ± 15.0	+ 2.33
Combined (Comb)	315 ± 19.3	+ 5.10	377 ± 22.3	+ 26.0

TABLE 4.23a: Comparison of means of TNPS of G in O. sanex senex with reference to stress conditions presented in Table 4.23.

F = 1934.62

CD = 25.22

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	NS	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	NS	NS	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.24: Effect of individual and combined in vivo stress of Cd & pH on TL of G in O. senex senex.

(Values, expressed as mg/g wet weight, are mean \pm S.D. of 6 determinations).

Control : 66.8 \pm 1.39

Stress	15 d	Change % Control	30 d	Change % Control
Cd	49.2 \pm 1.52	- 26.3	60.3 \pm 1.13	- 9.64
pH	17.5 \pm 1.16	- 73.7	36.6 \pm 1.34	- 45.1
Combined (Comb)	96.5 \pm 1.21	+ 44.5	68.6 \pm 1.15	+ 2.74

TABLE 4.24a: Comparison of means of TL of G in O. senex senex with reference to stress conditions presented in Table 4.24.

F = 15902.83

CD = 1.50

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.25: Effect of individual and combined in vivo stress of Cd & pH on ECP of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 537 \pm 16.6

Stress	15 d	Change % Control	30 d	Change % Control
Cd	413 \pm 13.7	- 23.7	688 \pm 22.6	+ 28.0
pH	384 \pm 12.9	- 28.5	474 \pm 22.5	- 11.8
Combined (Comb)	372 \pm 12.9	- 31.8	166 \pm 9.75	- 69.1

TABLE 4.25a: Comparison of means of ECP of haemolymph
in O. senex senex with reference to
stress conditions presented in Table 4.25.

F = 5364.65

CD = 19.40

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE * 4.26: Effect of individual and combined in vivo stress of Cd & pH on TAEAPS of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 69.0 \pm 5.02

Stress	15 d	Change % Control	33 d	Change % Control
Cd	32.7 \pm 5.85	- 52.6	47.6 \pm 9.03	- 31.0
pH	42.9 \pm 7.20	- 37.7	51.7 \pm 8.50	- 25.0
Combined (Comb)	85.5 \pm 2.56	+ 24.0	37.3 \pm 7.55	- 46.0

TABLE 4.26a: Comparison of means of TAEAPS of haemolymph in O. senex senex with reference to stress conditions presented in Table 4.26.

F = 455.4

CD = 8.02

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	NS	S	NS
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.27. Effect of individual and combined in vivo stress of Cd & pH on TAPAPS of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 33.2 ± 7.24

Stress	15 d	Change % Control	30 d	Change % Control
Cd	29.0 ± 5.83	- 12.7	41.7 ± 7.30	+ 25.6
pH	30.9 ± 6.28	- 6.90	35.6 ± 6.08	+ 7.20
Combined (Comb)	22.2 ± 2.12	- 33.2	17.3 ± 1.37	- 47.8

TABLE 4.27a: Comparison of means of TAPAPS of haemo-
lymph in O. senex senex with reference to
stress conditions presented in Table 4.27.

F = 211.73

CD = 6.60

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	S	S	NS	S
Cd 15	-	NS	S	S	S	S
pH 15	-	-	S	S	NS	S
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 4.28: Effect of individual and combined in vivo stress of Cd & pH on TP content of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 2534 \pm 329

Stress	15 d	Change % Control	30 d	Change % Control
Cd	2185 \pm 258	- 13.7	3137 \pm 496	+ 23.8
pH	2260 \pm 207	- 10.8	2715 \pm 285	+ 7.14
Combined (Comb)	2403 \pm 313	- 5.17	1964 \pm 164	- 22.5

TABLE 4.28a: Comparison of means of TP content of haemolymph in O. senex senex with reference to stress conditions presented in Table 4.28.

F = 450.5

CD = 362.52

Comparison of	. With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	NS	S	NS	S
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	NS	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.29: Effect of individual and combined in vivo stress of Cd & pH on TNPS of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 30.6 \pm 6.50

Stress	15 d	Change % Control	30 d	Change % Control
Cd	15.8 \pm 2.94	- 48.2	10.5 \pm 1.88	- 65.7
pH	23.0 \pm 4.88	- 24.8	14.8 \pm 4.00	- 51.4
Combined (Comb)	21.9 \pm 4.22	- 28.3	29.7 \pm 6.00	- 3.0

TABLE 4.29a: Comparison of means of TNPS of haemolymph in O. senex senex with reference to stress conditions presented in Table 4.29.

F = 161.7

CD = 5.38

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	NS
Cd 15	-	S	S	NS	NS	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 4.30: Effect of individual and combined in vivo stress of Cd & pH on TL of haemolymph in O. senex senex.

(Values, expressed as mg/100 ml, are mean \pm S.D. of 6 determinations).

Control : 533 \pm 81.6

Stress	15 d	Change % Control	30 d	Change % Control
Cd	983 \pm 75.3	+ 84.4	1333 \pm 103	+ 150
pH	933 \pm 103.3	+ 75.0	1450 \pm 105	+ 172
Combined (Comb)	1316 \pm 75.3	+ 146	1766 \pm 81.7	+ 231

TABLE 4.30a: Comparison of means of TL content of haemolymph in O. senex senex with reference to stress conditions presented in Table 4.30.

F = 1333.4

CD = 105.7?

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	NS	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not significant.

CD Value was calculated according to the formula given in Table 3.1a.

- Fig. 4.1: Percent change of total acid extractable anthrone positive substances (TAEAPS) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.2: Percent change of total acid precipitable anthrone positive substances (TAPAPS) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.3: Percent change of total protein (TP) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.4: Percent change of soluble protein (SP) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:4.1

CTGM TAEAPS

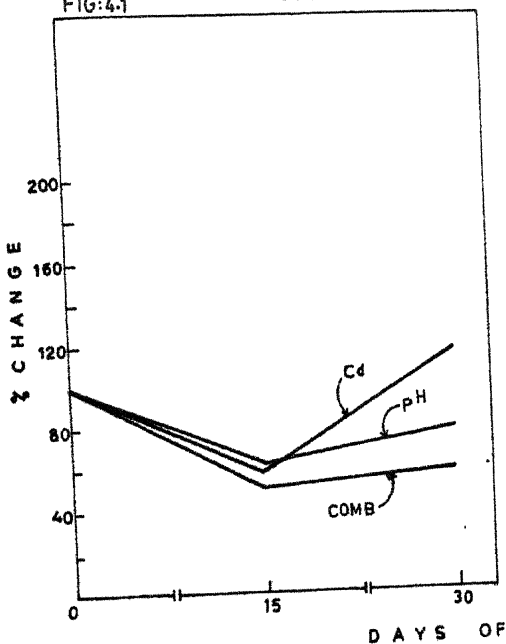


FIG 4.2

CTGM TAPAPS

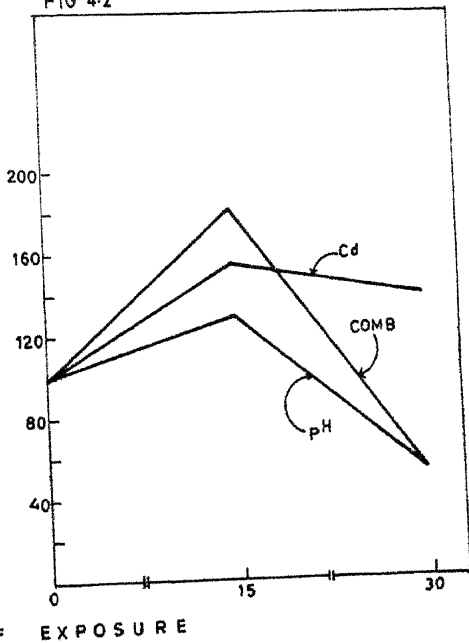


FIG:4.3

CTGM TP

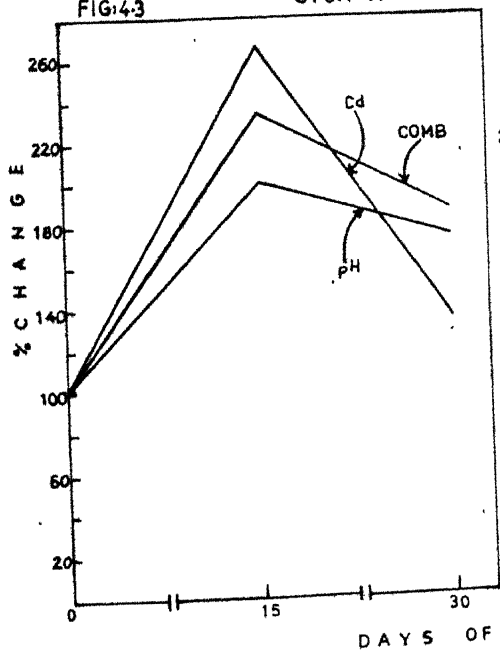


FIG:4.4

CTGM SP

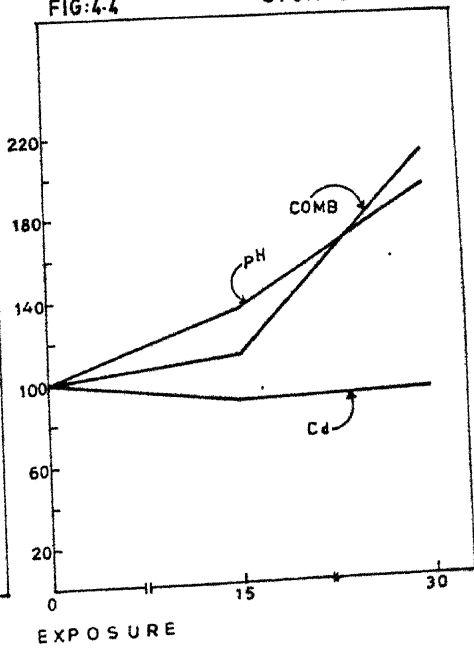


Fig. 4.5: Percent change of total ninhydrin positive substances (TNPS) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 4.6: Percent change of total lipid (TL) content in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and combinationally (Comb) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 4.7: Percent change of total acid extractable anthrone positive substances (TAEAPS) content in muscle (M) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 4.8: Percent change of total acidprecipitable anthrone positive substances (TAPAPS) content in muscle (M) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:4-5

CTGM TNPS

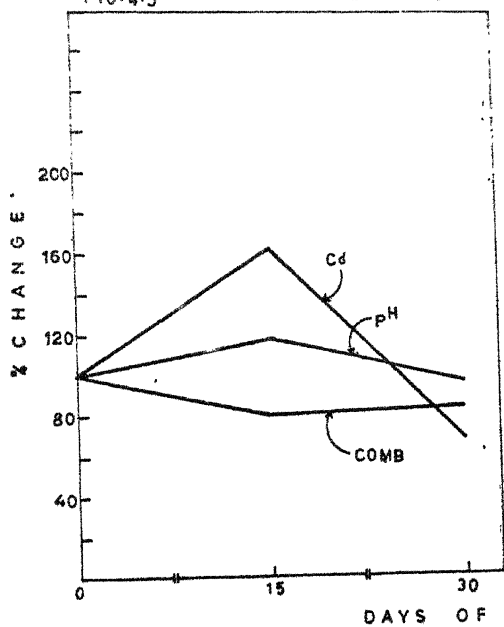


FIG 4 6

CTG TL

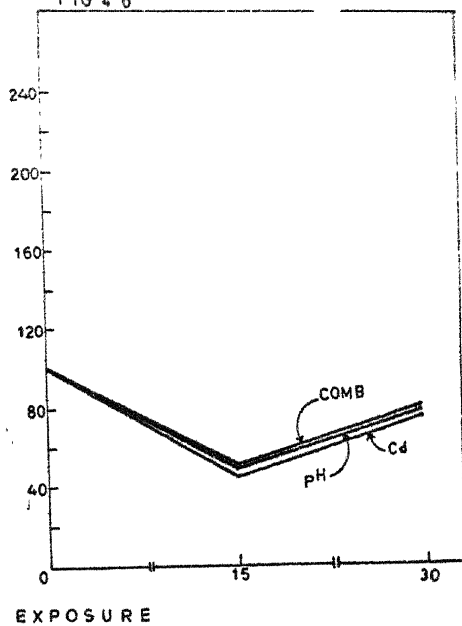


FIG:4-7

M TAEAPS

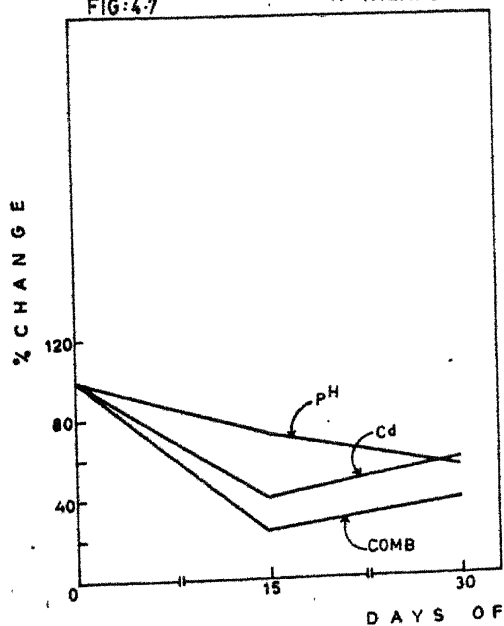
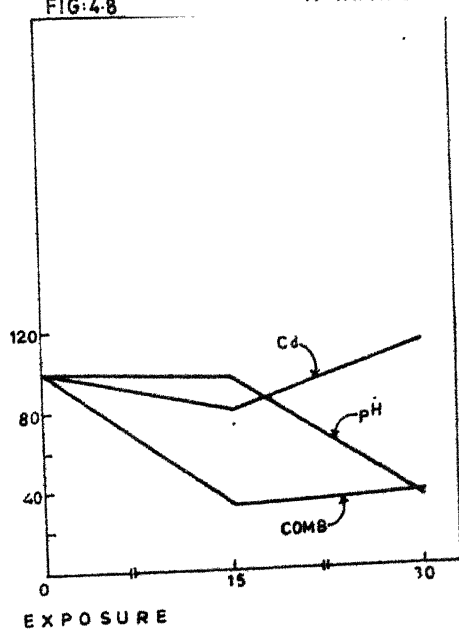


FIG:4-8

M TAPAPS



- Fig. 4.13: Percent change of total acidextrac-
table anthrone positive substances
(TAEAPS) content in hepatopaneas
(HP) of Cd-, pH- and combinationally
(Comb.) intoxicated crabs at 15 d
and 30 d sublethal exposure periods.
- Fig. 4.14: Percent change of total acid preci-
pitabile anthrone positive substances
(TAPAPS) content in hepatopaneas
(HP) of Cd-, pH- and combinationally
(Comb.) intoxicated crabs at 15 d and
30 d sublethal exposure periods.
- Fig. 4.15: Percent change of total protein (TP)
content in hepatopaneas (HP) of
Cd-, pH- and combinationally (Comb.)
intoxicated crabs at 15 d and 30 d
sublethal exposure periods.
- Fig. 4.16: Percent change of soluble protein
(SP) content in hepatopaneas (HP)
of Cd-, pH- and combinationally
(Comb.) intoxicated crabs at 15 d
and 30 d sublethal exposure periods.

FIG:4-13

HP TAEAPS

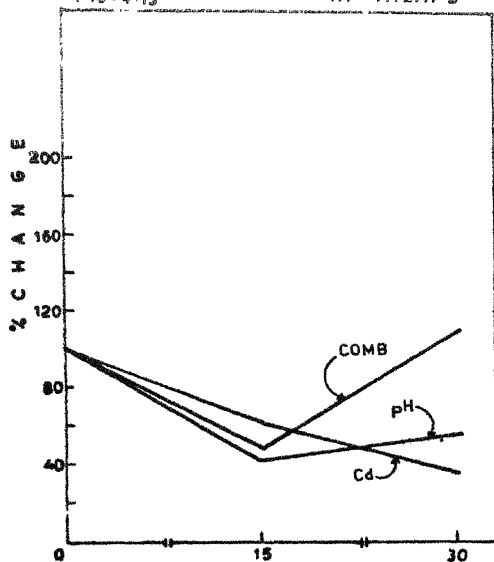


FIG:4-14

HP TAPAPS

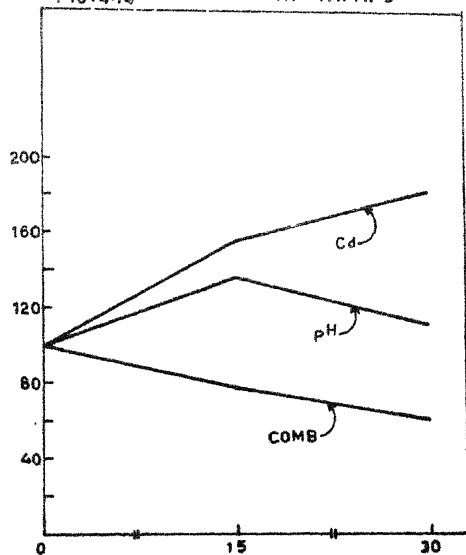


FIG:4-15

HP TP

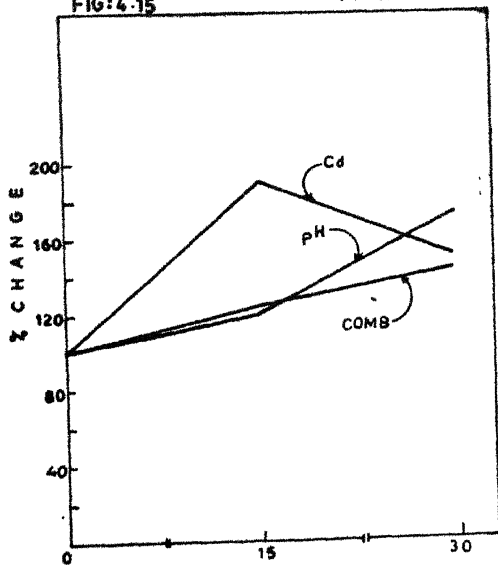
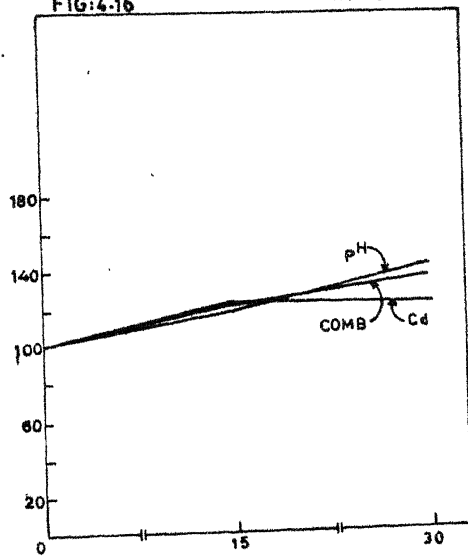


FIG:4-16

HP SP



- Fig. 4.17: Percent change : of total ninhydrin positive substances (TNPS) content in hepatopancreas (HP) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.18: Percent change of total lipid (TL) content in hepatopancreas (HP) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.19: Percent change of total acid extractable anthrone positive substances (TAEAPS) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.20: Percent change of total acid precipitable anthrone positive substances (TAPAPS) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:4.17

HP TNPS

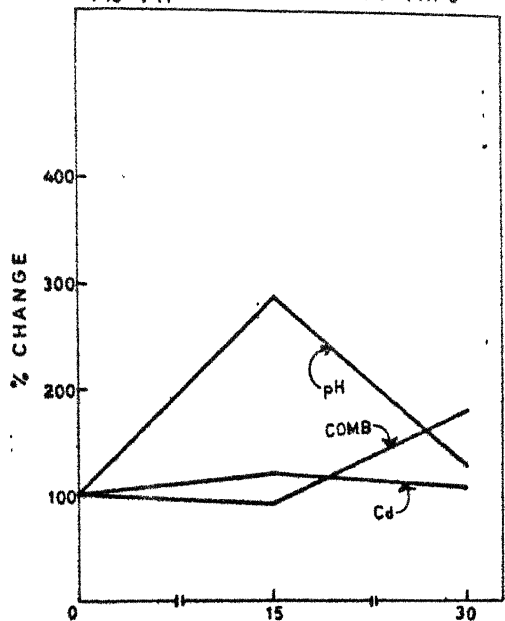


FIG:4.18

HPTL

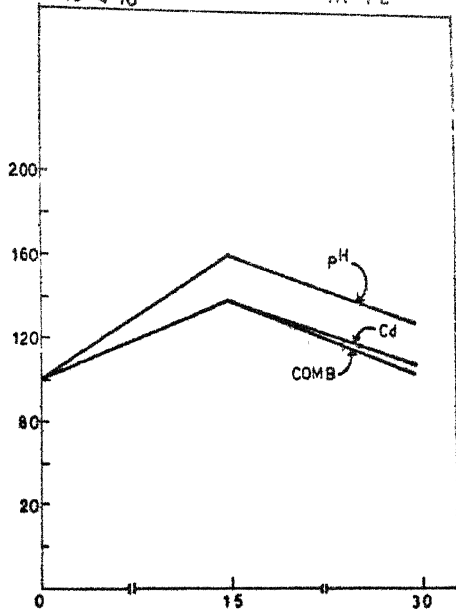


FIG:4.19

G TAEAPS

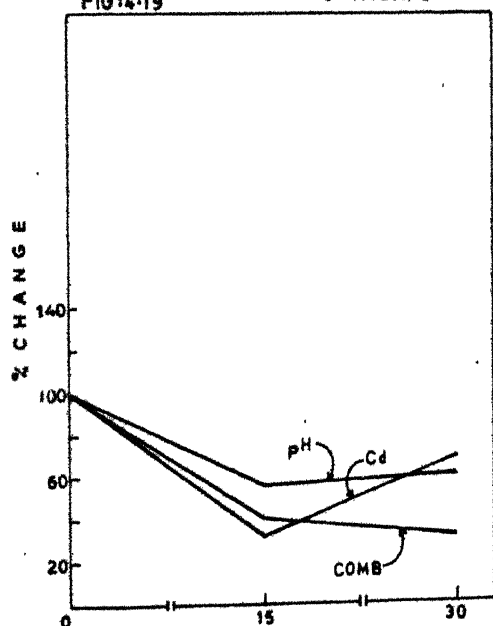
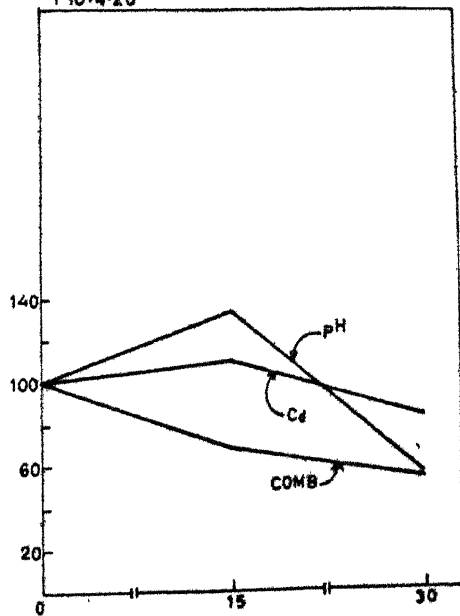
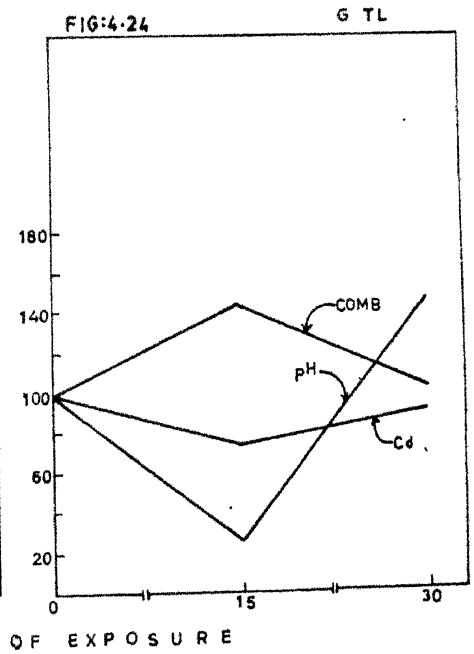
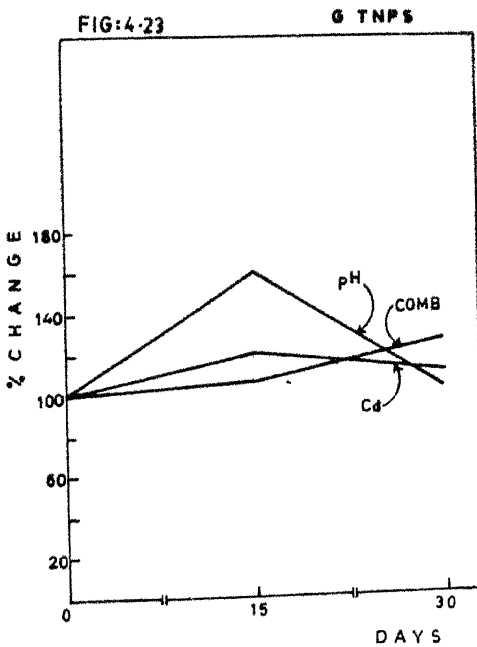
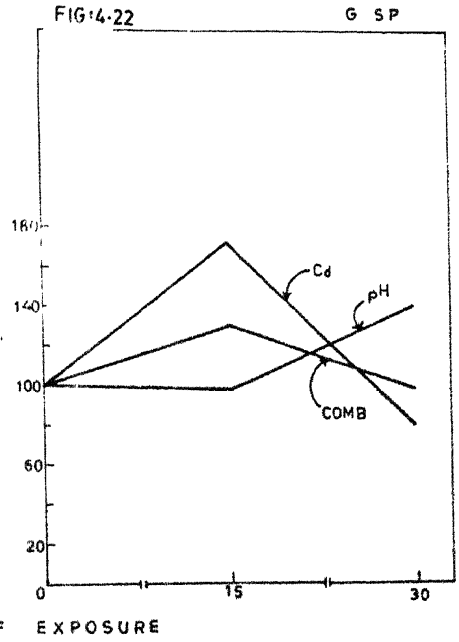
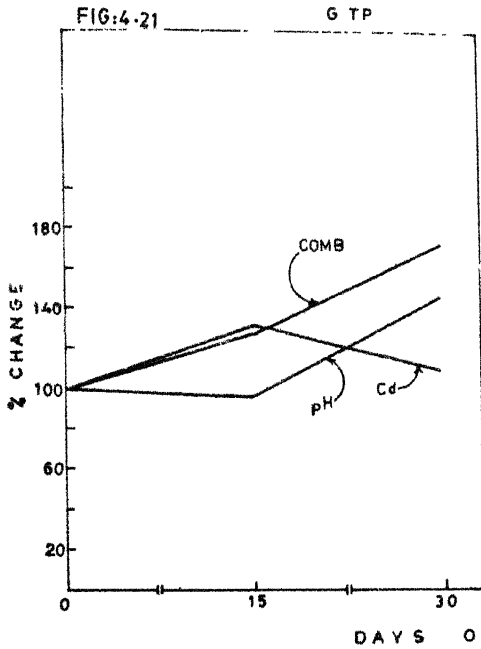


FIG:4.20

G TAPAPS



- Fig. 4.21: Percent change of total protein (TP) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.22: Percent change of soluble protein (SP) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.23: Percent change of total ninhydrin positive substances (TNPS) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.24: Percent change of total lipid (TL) content in gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.



- Fig. 4.25: Percent change of extra-cellular protein (ECP) in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.26: Percent change of total acid extrac-table anthrone positive substances (TAEAPS) in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.27: Percent change of total acid precipitable anthrone positive substances (TAPAPS) in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 4.28: Percent change of total protein (TP) content in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:4-25

HL ECP

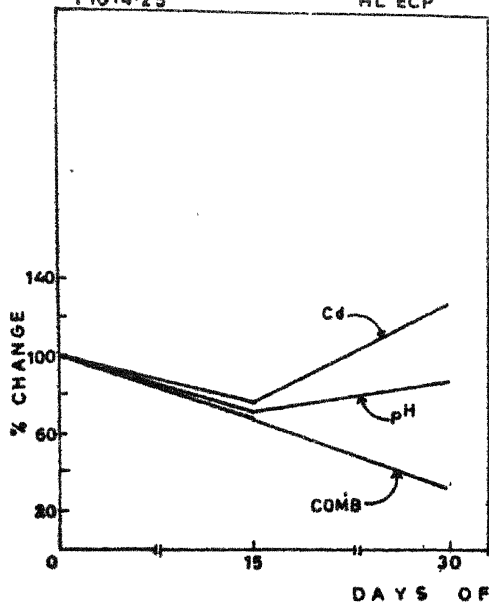


FIG:4-26

HL TAEAPS

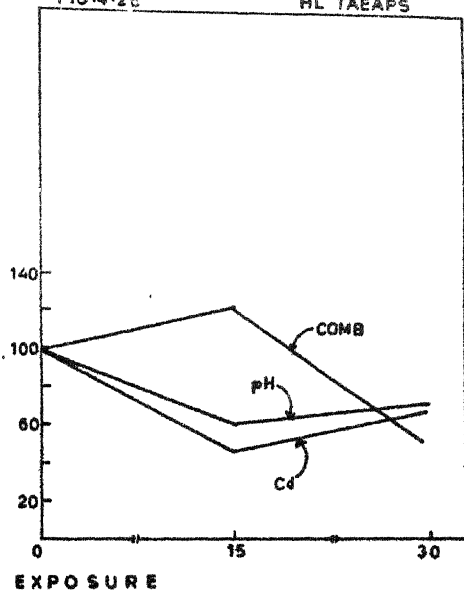


FIG:4-27

HL-TAPAPS

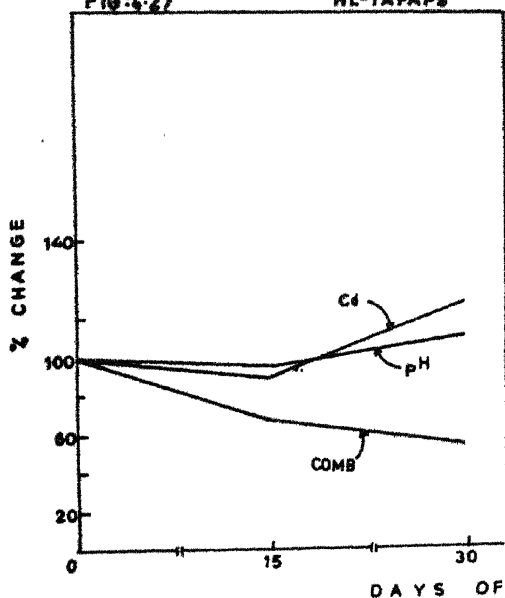


FIG:4-28

HL-TP

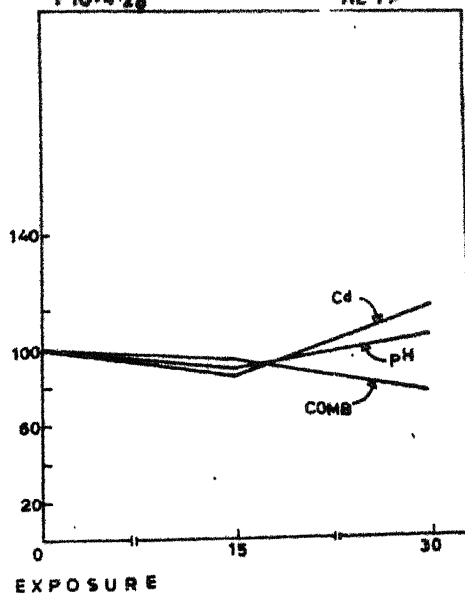


Fig. 4.29: Percent change of total ninhydrin positive substances (TNPS) in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 4.30: Percent change of total lipid (TL) in haemolymph (HL) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:4.29

HL-TNPS

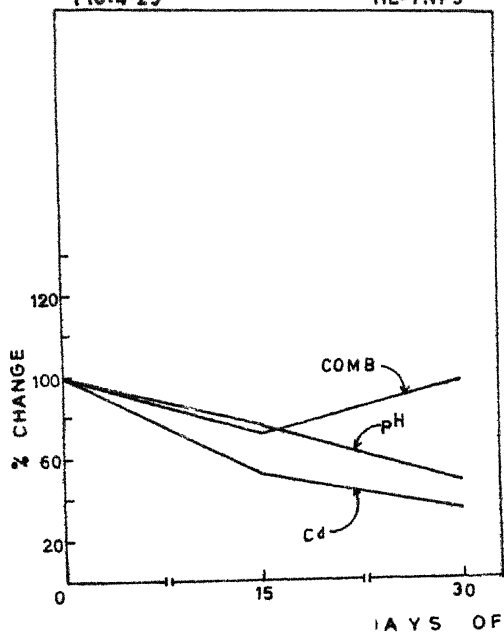
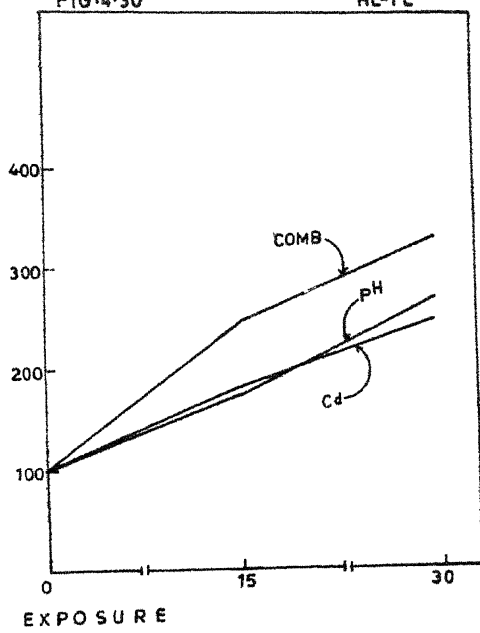


FIG:4.30

HL-TL



CHAPTER V

ACTIVITY LEVELS
OF ENZYMES

The stressants Cd and pH
have been shown as toxic
agents prima facie (Chapter
II), affecting the metabo-

V 1 INTRODUCTION

lism of the organism, O. senex senex (Chapter III). They
have been found to inflict alterations in the organic
composition of the tissues of the crab (Chapter IV). All
these experimental data reflect the influence of the stre-
ssants on the sub-cellular catalytic machinery viz., the
enzyme systems.

In this chapteral location, data on the activity
levels of some pertinent enzymes with reference to Cd and
pH stresses, in the crab will be presented.

In the present work, the
following enzymes were
selected keeping in view
the physiological and organic
compositional alterations

V 2 MATERIALS AND METHODS

V 2.A ENZYME ASSAYS

caused by the stressants in the organisms.

1) AChE:

Intoxication by stressants is generally known
to include impairment of locomotor physiology through the
breakdown of the nervous function. The functional inte-

grity of nervous system is reflected in the activity of the enzyme acetyl choline esterase (AChE). In toxicological investigational protocol, AChE activity of the neural tissue is included as an important parameter. In the present work, the activity of AChE was estimated in the cephalothoracic ganglionic mass (CTGM) under the individual and combinational regimes of the stressants Cd and pH.

The assay protocol was according to Metcalf et al., (1957).

The reaction mixture in a final volume of 2 ml contained: 100 μ moles of phosphate buffer (0.2M); 8 μ moles of substrate (acetyl choline chloride) of 0.2 ml of crude homogenate (enzyme source). The reaction was initiated by the addition of enzyme source and the reaction mixture was incubated for 30 minutes at the ambient temperature. The reaction was arrested by the addition of 2 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 1:1 HCl: water. The contents were shaken well and centrifuged at 1400 n_{pm} . To the supernatant 0.5 ml of 10% ferric chloride solution was added and the optical density was measured immediately at 540 nm in Bausch and Lomb Spectronic 20 colorimeter against reagent blank. Zero time controls were maintained along with endogenous blanks. Protein

content was estimated following the procedure of Lowry et al., (1951).

2) ATPase activity

To assess the effect of stressants on the energetics of the tissue, the activity levels of ATPase in the tissues of O. senex senex were assayed under the individual and combinational stress of Cd and pH.

The assay protocol for ATPase was fashioned according to Tirri et al., (1973), with slight modification.

2% homogenates of the tissues were prepared in ice-cold 0.25M sucrose solution containing 0.001M EGTA and 0.01M imidazole. The homogenate was divided into 2 parts. One part was centrifuged at 1400~~rpm~~ and the supernatant thus obtained was used as enzyme source for Mg^{2+} -ATPase. The second uncentrifuged portion was used as the source for the assay of total ATPase.

The constitution of reaction mixture was arrived at following basic assay protocol studies.

Total ATPase

The reaction mixture in a final volume of 2 ml contained 100 μ moles of Tris buffer; 20 μ moles each of

MgCl₂, NaCl and KCl (cofactors); substrate i.e., disodium ATP (8 μ moles).

Mg²⁺-ATPase

Reaction mixture in a final volume of 2 ml contained: 100 μ moles of Tris buffer; 20 μ moles of MgCl₂; 5 μ moles of NaCl, 5 μ moles of KCl; 10 μ moles of ouabain (potent inhibitor of Na⁺-K⁺-ATPase); 8 μ moles of substrate (disodium ATP).

The optical density measurements were made in Bausch and Lomb Spectronic 20 colorimeter at 720 nm against reagent blank.

The protein content was estimated 'Folinometrically' (Lowry et al., 1951).

3) Activity levels of dehydrogenases:

The energetic status of tissues is a function of not ATPases alone. The dehydrogenase system of the tissues forms the primary biochemical cistern of the tissue 'energetic-drain' system. In the present work the activity levels of 4 dehydrogenases were estimated in the tissues of the normal and stressant treated animals.

Assay Methods

The activity level of lactate dehydrogenase (LDH; L-lactate: NAD oxidoreductase EC.1.1.1.27) was assayed according to King (1965). The activity level of succinate dehydrogenase (SDH; succinate: acceptor oxidoreductase EC.1.3.99.1) was according to Nachlas et al., (1960). The assay of glutamate dehydrogenase (GDH; glutamate: NAD oxidoreductase EC.1.4.1.3) was according to Lee and Lardy (1965) and the malate dehydrogenase (MDH; malate: NAD oxidoreductase EC.1.1.37) assay was according to Nachlas et al., (vide ut supra).

The tissue homogenates, prepared in ice-cold 0.25M sucrose were centrifuged for 15 minutes at 2500 rpm. The supernatants were used as enzyme sources

After assessment of basic assay protocol, the following assay composition was arrived at as 'standardized' assay mixture, for the different enzymes.

'Standardized' mixtures for dehydrogenases

Enzyme assayed	Buffer μmoles	Substrate* μmoles	Enzyme source ml	INT μmoles	NAD μmoles
LDH	100	40	0.3 (0.5)**	2.0	0.1
SDH	100	40	0.3 (0.5)**	2.0	0.1
GDH	100	40	0.3 (0.5)**	2.0	0.1
MDH	100	40	0.3 (0.5)**	2.0	0.1

* Sodium salt of the relevant substrate was used

** Values in parentheses give the volume of homogenate supernatant used for muscle tissue.

The reaction was initiated by the addition of enzyme source and the reaction mixture was incubated for 30 minutes at the ambient temperature. The reaction was stopped by the addition of 5 ml of glacial acetic acid to the reaction mixture. The formazan formed was extracted overnight in 5 ml of toluene at 5°C. The optical density due to formazan was read at 495 nm in a Bausch and Lomb Spectronic 20 colorimeter, against toluene blank. Protein content of enzyme source was estimated according to Lowry et al., (1951).

4. Activity levels of Aminotransferases:

The activity levels of the 2 major aminotransferases viz., aspartate (AAT, L-aspartate: 2-oxoglutarate aminotransferase, E.C.2.6.1.1) and alanine (AlAT, DL-alanine: 2-Oxoglutarate aminotransferase, E.C.2.6.1.2) aminotransferase were assayed according to the method of Reitman and Frankel (1957).

The assays were carried on the sucrose homogenate supernatants.

The assays were carried out in three tissues viz., hepatopancreas (HP), cephalothoracic ganglionic mass (CTGM) and chelate leg muscle (M) of O. senex senex. The incubation mixture for the two aminotransferases was constituted as follows:

Composition of incubation mixture for aminotransferases

Enzyme	AAT	AlAT
-----	-----	-----
Additions		
^a Substrate	100 umoles	200 umoles
2-Oxoglutarate	2.5 umoles	2.5 umoles
^b Buffer	100 umoles	100 umoles
Enzyme source	Optimal quantum	Optimal quantum
Final volume	1.0 ml	1.0 ml

^aL-aspartate : for AAT

DL-alanine : for AlAT

^bPhosphate buffer: pH 7.4

Incubation time (at lab.temp.) for AAT: 60 minutes;
for AlAT: 30 minutes; pyruvate determined colorimetrically
with Bausch and Lomb Spectronic-20 colorimeter against
blank, at 546 nm.

The stressant regimes, and
V 2.B PRELIMINARY durations used for experimen-
STANDARDIZATION tation are given elsewhere
(Chapter IV). Tissues for isolation from the organisms
have already been listed out (Chapter II). From the
organisms, the tissues were isolated in cold.

Homogenization of the tissue was carried out
in cold in 0.25M sucrose medium. The homogenate was
centrifuged at 2400 rpm and the supernatant was used
as the 'enzyme source'. This enzyme source was used
in the enzyme assay studies.

In preliminary standardization experiments, optimal values were determined for the quantum of tissue, substrate etc., for preparation of incubation mixtures. These standardized values were employed in the subsequent systematic 'enzymometric' experiments.

The data with regard to tissue quantum used in incubation mixture are appended below:

S.No.	Tissue	Quantum
1.	Cephalothoracic ganglionic mass (CTGM)	30 mg
2.	Hepatopancreas (HP)	20 mg
3.	Chelate leg muscle (M)	20 mg
4.	Gill (G)	20 mg

The optimal pH condition for the different enzymes employed in the present work are shown below:

S.No.	Enzyme	Optimal pH
1.	AChE	7.4
2.	ATPase	7.6
3.	Dehydrogenases	7.4
4.	Transaminases	7.4

Since the present crab is poikilothermic or ectothermic, the enzyme assay was carried out at the ambient temperature prevailing in the laboratory at the time of experimentation. The temperature varied between 29-34°C.

V 3 RESULTS

V 3.A CTGM

The activity levels of AChE in the cephalothoracic ganglionic mass (CTGM) are given in table 5.1 and fig. 5.1. The results of statistical assessment of these data are given in table 5.1a. The data given in table 5.1 show that the stressants show diverse effects on the AChE system of the tissue which are found to involve statistically significant variation (Table 5.1a).

The variation of AChE activity level is generally in the positive (incremental) direction.

Cd-stress causes an shorter-duration elevation (+ 10.8% control) which is not statistically significant. The longer stress-duration under Cd-regime also registers a slightly higher elevation (+ 17.0% control, 30 days post-stress (dps)) of AChE activity, which is not statistically significant.

pH-stress causes a statistically significant depression during the shorter stress-duration (- 28.0% control, 15 dps) and elevation during longer stress-duration (+ 32.3% control, 30 dps).

Under combinational regime, the activity level of the enzyme is slightly and non-significantly modified during shorter stress-duration (+ 4.6% control, 15 dps)

and shows a very remarkable elevation during the longer stress-duration (+ 177% control, 30 dps).

The stressant regimes cause diverse effects on the total ATPase activity levels of CTGM (Table 5.2; Fig. 5.2). Statistical analysis of the data are given in table 5.2a.

Cd-stress causes a shorter stress-duration elevation (+ 12.2% control, 15 dps) of the activity level of the enzyme which is significant statistically; during longer stress-duration, the stress causes a depression (- 28.0% control, 30 dps) which too is statistically significant.

Under pH-stress, during shorter stress-duration the activity level of ATPase is elevated (+ 37.8%, control, 15 dps), statistically significant; during longer stress-duration, a depression is noted (- 27.2% control, 30 dps) which is statistically significant.

Under the combinational regimes the activity level of enzyme shows elevation. During the shorter stress-duration, the elevation is small (+ 3.5% control 15 dps) and statistically non-significant; during longer stress-duration an astonishingly high elevation is noted (+ 412% control, 30 dps).

The stressant regimes impose divergent variations on the activity level of Mg^{2+} -ATPase enzyme (Table 5.3; Fig. 5.3). Statistical treatment of the data is given in table 5.3a.

Under the individual Cd-regime, the activity level of the enzyme undergoes a shorter stress-duration elevation (+ 98.5% control, 15 dps) which is statistically significant and longer stress-duration depression (- 8.3% control, 30 dps) which is non-significant.

pH-regime also causes a shorter stress-duration elevation (+ 148% control, 15 dps) and a longer stress-duration depression (- 5.3% control, 30 dps),

Under combinational regime of the stressants, elevations are not during both stress-durations. The elevation during the longer stress-duration (+ 362% control, 30 dps) is astonishingly high and the shorter stress-duration elevation (+ 95.5% control, 15 dps) is also noteworthy.

The data given in table 5.4 and figure 5.4 depict the variable influence of the different stressant regimes on the activity levels of AAT in CTGM. Statistical treatment of the data is provided in table 5.4a.

Cd-regime causes small changes in the activity levels of the enzyme during both stress-durations (+ 2.0% control, 15 dps; + 4.0% control, 30 dps). These changes are not statistically significant.

pH-stress in contrast, causes remarkable and statistically significant alterations in the activity levels of this enzyme, AAT. While the shorter stress-duration depression (- 89.7% control, 15 dps) is noteworthy, the longer stress-duration elevation is astounding (+ 300% control, 30 dps).

Under combinational regime, the activity levels of AAT are modified in the positive (elevation) direction in both stress-durations. Of these elevations, the longer stress-duration elevation is astounding as in the case of pH-regime (+ 13.3% control, 15 dps; + 301% control, 30 dps).

The activity levels of the enzyme ALAT are modified diversely by the different stress combinations and durations (Table 5.5; Fig. 5.5). These modifications are found to comprise of statistically significant variance (Table 5.5a).

Cd-stress has a generally depressory effect on the ALAT activity levels of CTGM (- 13.5% control;

15 dps; - 30.0% control, 30 dps). The longer stress-duration effect is found to be statistically significant.

pH-stress shows diverse effect in the stress-durations. In the shorter stress-duration, a considerable quantum of depression (- 68.5% control, 15 dps) and in the longer duration of duress, an astonishing elevation (+ 403% control, 30 dps).

In the combination regime, the pH-regime pattern of effects is repeated, but at a slightly smaller scale (- 16.5% control, 15 dps; + 350% control, 30 dps).

Table 5.6 (Fig. 5.6) gives evidence for the general depressory effect of the different stressant regimes on the activity levels of GDH of CTGM in O. senex senex. The data are found to comprise of significant variance (Table 5.6a).

Cd-stress causes depression of the activity level of GDH in both stress-durations (- 75.0% control, 15 dps; - 61.3% control, 30 dps). These changes are statistically significant.

The modification pattern under pH-regime is similar to that under Cd-regime (- 56.0% control, 15 dps; - 45.4% control, 30 dps). Changes under this regime also are statistically significant.

Under the combinational regime, the activity level of GDH undergoes a shorter stress-duration depression (- 17.7% control, 15 dps; statistically non-significant) and longer stress-duration elevation (+ 15.4% control, 30 dps; statistically non-significant).

The activity level of SDH of CTGM is uniformly depressorily modified by the different stressant-regimes (Table 5.7; Fig. 5.7). These alterations are found to comprise of statistically significant variance (Table 5.7a).

Under the three regime-types, the depressory effects are progressive i.e., smaller in the shorter stress-duration and larger in the longer stress-duration (Cd: - 61.8% control, 15 dps; - 53.3% control, 30 dps; pH: - 48.8% control 15 dps; - 74.5% control, 30 dps; combinational regime: - 19.4% control, 15 dps; -53.3% control, 30 dps).

The activity levels of LDH of CTGM is modified in a general depressory direction under the different stressant regimes (Table 5.8; Fig. 5.8; Statistical evaluation: Table 5.8a).

Under Cd-stress, the activity level of LDH is consistently depressed (- 53.8% control, 15 dps;

-61.0% control, 30 dps). The situation is the same under pH-stress too (- 79.4% control, 15 dps; - 42.8% control, 30 dps).

Under the combinational regime, the pattern of modification of enzyme activity is made of depression-elevation sequence i.e., shorter stress-duration depression and longer stress-duration elevation (- 71.8% control; 15 dps; + 91.0% control, 30 dps).

Under the different stressant regimes, the activity level of MDH of CTGM of O. senex senex shows a depression-elevation pattern, with reference to the two stress-durations employed (Table 5.9; Fig. 5.9; Statistical evaluation: Table 5.9a).

Under Cd-stress, the shorter stress-duration depression of activity level of the enzyme (- 45.0% control, 15 dps) is statistically significant while the longer stress-duration (+ 11.0% control, 30 dps) is not. The situation is similar under pH-stress (15 dps: - 21.3% control, significant; 30 dps: + 4.0% control, not significant).

Under combinational regime however the alterations in both stress-durations are statistically significant; the shorter stress-duration depression (- 41.0% control, 15 dps) is considerable while the longer stress-

duration elevation (+ 168% control, 30 dps) is more remarkable.

V 3.B MUSCLE

The variations in the activity level of AChE in the chelate leg muscle (M) of O. senex senex under the different stress regimes are given in table 5.10 (Fig. 5.10; Statistical evaluation; Table 5.10a). The trend of variation is generally on the elevatory side.

Under Cd-stress a progressive elevatory pattern is noted (15 dps: 103% control; 30 dps: 183% control). pH-stress induced alterations follow elevation: depression pattern (15 dps: + 74.5% control, 30 dps: - 27.5% control).

Under combinational stress, shorter stress-duration causes a small and statistically non-significant elevation (+ 3.2% control, 15 dps) while the longer stress-duration causes a remarkable elevation (+ 107% control, 30 dps).

Chelate leg muscular total ATPase activity level is found to be consistently elevated under different stressant regimes (Table 5.11; Fig. 5.11; Statistical evaluation: Table 5.11a).

Under Cd-stress, the positive change of enzyme activity, the 'conservatory' pattern: higher elevation in shorter stress-duration being followed by smaller elevation in the longer stress-duration (15 dps: +64.5% control; 30 dps: + 26,5% control). Under pH-regime also the same pattern is obtained (15 dps: + 81.0% control; 30 dps: + 8.4% control).

Under the combinational regime the shorter stress-duration shows small, statistically non-significant elevation (+ 5.0% control, 15 dps) while the longer stress-duration shows a very remarkable elevation (+165% control, 30 dps).

Under the different stress regimes, a general trend of elevation of the activity level of Mg^{2+} -ATPase is discernible (Table 5.12; Fig. 5.12; Statistical evaluation: Table 5.12a).

Under Cd-stress regime, the pattern is of the elevation: depression type (15 dps: + 44.0% control; 30 dps: - 2.8% control). Under pH-regime the pattern is conservatory elevation (15 dps: + 143% control; 30 dps: + 106% control). Under the combinational regime the pattern is progressive elevation (+ 124% control, 15 dps; + 186% control, 30 dps).

The activity level of AAT in M of O. senex senex under the different stressant regimes shows a consistent elevatory picture (Table 5.13; Fig. 5.13; Statistical evaluation: Table 5.13a).

Under Cd-regime, the pattern is conservatory elevation (+ 75.0% control, 15 dps; + 57.5% control, 30 dps). Under pH-and combinational regimes also similar trends are noted (pH-regime: 15 dps: + 30.0% control; 30 dps: + 333% control; combinational regime: + 150% control, 15 dps; + 275% control, 30 dps).

The activity level of ALAT also shows a consistent elevatory picture in the chelate leg muscular tissue of O. senex senex under the different stressant regimes (Table 5.14; Fig. 5.14; Statistical evaluation: Table 5.14a).

Under Cd-regime the pattern is conservatory elevation (15 dps: + 113% control; 30 dps: + 8.2% control). Under pH-regime the pattern is progressive elevation (15 dps: + 7.0% control; 30 dps: + 142% control). Under combinational regime also a progressive elevation pattern is noted (15 dps: + 109% control; 30 dps: +274% control).

The activity level of glutamate dehydrogenase in the chelate leg muscle of O. senex senex undergoes con-

sistently depressory alterations under the influence of the different stressant regimes (Table 5.15; Fig. 5.15; Statistical evaluation, Table 5.15a).

Cd-stress cause a conservative depressory changes in the activity level of the enzyme (15 dps: - 71.2% control; 30 dps: -44.5% control).

pH-stress, on the other hand, causes a progressive depressory change (15 dps: - 49.5% control; 30 dps: -80.0% control).

Under the combinational regime, the change is small, and has 'conservatory' 'profile' (15 dps: - 16.7% control; 30 dps: - 2.8% control).

The activity level of succinate dehydrogenase of muscle of O. senex senex presents a picture of general depression under the diverse stressant regimes (Table 5.16; Fig. 5.16; Statistical evaluation: Table 5.16a).

Under Cd-regime, the pattern is of conservatory depression (- 74.5% control, 15 dps; - 62.3% control, 30 dps). Under pH-regime, the pattern is reversed (- 52.8% control, 15 dps; - 87.5% control, 30 dps).

Under the combinational regime, the pattern is depression-elevation (- 28.0% control, 15 dps; + 46.2% control, 30 dps).

Under the different stressant regimes, the activity level of lactate dehydrogenase undergoes consistent depression in the muscle tissue of O. senex senex (Table 5.17; Fig. 5.17; Statistical evaluation: Table 5.17a).

The changes under Cd-stress show conservative depression pattern (15 dps: - 52.8% control; 30 dps: - 20.0% control).

Under pH-stress a reversed pattern is seen (15 dps: -30.0% control; 30 dps: - 92.0% control). Under combinational stress the pattern of pH-stress is repeated i.e., that of progressive depression (15 dps: - 4.8% control; 30 dps: - 56.4% control).

Table 5.18 gives data on the effect of different stressant regimes on the activity levels of malate dehydrogenase of the chelate leg muscle of O. senex senex (please see Fig. 5.18 for graphical visualization of the trends portrayed by the data; Statistical evaluation: Table 5.18a).

Under Cd-stress, the pattern of progressive depression is evident (- 7.0% control, 15 dps; - 27.0% control, 30 dps). Under pH-stress the picture of change is that of elevation (slight); depression (+ 6.6% control, 15 dps; - 53.7% control, 30 dps). Under combina-

tional stress the picture is that of elevation: depression (small) (+ 35.8% control, 15 dps: - 7.0% control, 30 dps).

V 3.C HEPATOPANCREAS
(MIDGUT GLAND)

The activity levels of total ATPase in the hepatopancreas (midgut gland) of O. senex senex under diverse stressant regimes are given in

table 5.19 (Fig. 5.19; Statistical evaluation: Table 5.19a).

Elevation: depression pattern is perceptible in the total ATPase activity of HP under Cd-regime (+ 94.0% control, 15 d₁s; - 35.8% control, 30 dps).

Under pH-regime also a similar pattern is evident (+ 5.0% control, 15 dps; -50.0% control, 30 dps).

Under combinational regime, the shorter stress-^(small)duration leads to a 9.9% elevation / of total ATPase activity whereas the longer stress-duration leads to a very remarkable elevation of the enzyme activity (+148% control, 30 dps).

Table 5.20 and Fig. 5.20 pertain to the data on the influence of the stressant regimes on the hepa-

topancreatic Mg^{2+} -ATPase activity in O. senex senex
(Statistical evaluation: Table 5.20a).

Under Cd-stress the pattern is elevation: depression (+ 113% control, 15 dps; - 21.5% control, 30 dps). Under pH-stress also a similar pattern is observable (+ 67.4% control, 15 dps; - 36.8% control, 30 dps). Under combinational regime the pattern of progressive elevation is evident (+ 45.3% control, 15 dps ; + 81.3% control, 30 dps).

Under the diverse stressant regimes, the activity levels of aspartate aminotransaminase in the hepatopancreas of O. senex senex conform to a pattern of consistent elevation (Table 5.21; Fig. 5.21; Statistical evaluation: Table 5.21a).

Under Cd-regime the pattern is conservatory elevation in which the shorter stress-duration elevation is astounding (+ 364% control, 15 dps; + 19.4% control, 30 dps).

Under pH-regime a reversed trend is evident, the longer stress-duration elevation being very remarkable (+ 5.8% control, 15 dps; + 248% control, 30 dps). Under the combinational regime, the pH-regime pattern is repeated (+ 65.7% control, 15 dps; + 248% control, 30 dps).

Table 5.22 and fig. 5.22 give a picture of changes induced by the diverse stressant regimes in the activity level of alanine aminotransferase of hepatopancreas of O. senex senex (Statistical evaluation: Table 5.22a).

Under Cd-stress regime a conservatory elevation is evident (+ 343% control, 15 dps; + 17.0% control, 30 dps). Under pH-regime, the pattern depression: elevation (- 11.5% control, 15 dps; +396% control, 30 dps). Under combinational regime the pattern is progressive elevation (+ 77.0% control, 15 dps; + 290% control, 30 dps).

Under the different stressant regimes, the activity level of glutamate dehydrogenase of hepatopancreas of O. senex senex is diversely modified (Table 5.23; Fig. 5.23; Statistical evaluation: Table 5.23a).

Under Cd-regime a picture of conservative elevation is evident (+ 291% control, 15 dps; + 54.5% control, 30 dps).

Under pH-regime a picture of progressive depression is evident. However, this provision is not steep and therefore is of a very small order (- 20.0% control, 15 dps; - 25.4% control, 30 dps).

Under the combinational regime, a depression: elevation pattern is evident (- 12.0% control, 15 dps; + 74.0% control, 30 dps).

The activity level of succinate dehydrogenase in the hepatopancreas of O. senex senex is positively (i.e., elevationally) modified, almost consistently under the different stressant regimes (Table 5.24; Fig. 5.24; Statistical evaluation: Table 5.24a).

Under Cd-stress a picture of conservatory elevation of enzyme activity is evident (+ 456% control, 15 dps; + 142% control, 30 dps). Under pH-regime, progressive elevation pattern is evident (+ 20.0% control, 15 dps; + 55.3% control, 30 dps).

Under combinational regime a pattern of depression: elevation is evident. These changes however are very small and statistically non-significant (- 3.3% control, 15 dps; + 9.1% control, 30 dps).

The hepatopancreatic lactate dehydrogenase activity in O. senex senex is modified in the positive (elevation) direction in general under the stressant regimes studied (Table 5.25; Fig. 5.25; Statistical evaluation: Table 5.25a).

Under Cd-regime a conservatory elevation is evident (+ 378% control; 15 dps; + 98.3% control, 30 dps). Under pH-regime, the activity level of enzyme shows elevation: depression pattern (+ 7.4% control, 15 dps; - 63.6% control, 30 dps). Under combinational regime progressive elevation pattern is evident (+7.4% control, 15 dps; + 65.3% control, 30 dps).

The hepatopancreatic malate dehydrogenase activity in O. senex senex is diversely modified by the different stressant-regimes (Table 5.26; Fig.5.26; Statistical evaluation: Table 5.26a).

Under Cd-stress the pattern of conservatory elevation is evident (+ 160% control, 15 dps; +33.6% control, 30 dps). Under pH-duress, depression: elevation pattern is seen (- 42.8% control, 15 dps; + 52.0% control, 30 dps). Under combinational regime also a similar pattern is evident. However, in this case, the longer stress-duration elevation is very remarkable (- 59.2% control, 15 dps; + 250% control, 30 dps).

V.3.D GULL The activity level of total
ATPase of gill of O. senex
senex under different stress-
regimes is affected diversely (Table 5.27; Fig. 5.27;
Statistical evaluation: Table 5.27a).

Cd-stress shows a picture of conservatory depression (- 40.0% control, 15 dps; - 29.5% control, 30 dps). Under pH-stress, elevation: depression pattern is evident (+ 22.0% control, 15 dps; - 56.6% control, 30 dps).

Under combinational regime, the activity level of enzyme gives a picture of progressive elevation (+ 8.5% control, 15 dps; + 79.0% control, 30 dps).

The activity level of branchial Mg^{2+} -ATPase in O. senex senex is modified generally in the depression direction under the different stressant regimes studied (Table 5.28; Fig. 5.28; Statistical evaluation: Table 5.28a).

Cd-regime shows a progressive depression pattern (- 29.5% control, 15 dps; - 50.0% control, 30 dps). pH-regime also shows a similar pattern (- 22.0% control, 15 dps; - 45.2% control, 30 dps).

Under combinational regime, the shorter stress-duration shows a very small positive change of the activity level of enzyme (+ 2.0% control, 15 dps). In longer stress-duration a positive change of notable magnitude occurs (+ 21.0%, control, 30 dps).

Table 5.29 and figure 5.29 give numerical and graphical accounts respectively, of the changes in the

activity level of branchial glutamate dehydrogenase in O. senex senex induced by different stressant regimes studied (Statistical evaluation: Table 5.29a).

Under the different regimes the enzyme activity is modified consistently in the negative (depression) direction.

The Cd-stress induced changes present a pattern of progressive depression (- 51.7% control, 15 dps; -60.0% control, 30 dps).

The pH-stress induced changes present a conservatory depression pattern (- 51.7% control, 15 dps; - 2.4% control, 30 dps).

The combination-regime induced changes present a progressive depression pattern (- 42.0% control, 15 dps; - 96.3% control, 30 dps).

Under the regimes of stressants studied, the branchial succinate dehydrogenase activity in O. senex senex presents a picture of consistent depression (Table 5.30; Fig. 5.30; Statistical evaluation: Table 5.30a).

Under all the regimes the changes are patterned as progressive depression (Cd-regime: - 47.2% control, 15 dps; - 65.7% control, 30 dps; pH-regime: + 2.0% control, 15 dps;

- 32.4% control, 30 dps; Combinational regime: - 57.0% control, 15 dps; - 85.0% control, 30 dps).

The activity level of branchial lactate dehydrogenase in O. senex senex under the different stressant-regimes presents a picture of consistent depression (Table 5.31; Fig. 5.31; Statistical evaluation: Table 5.31a)

Under Cd-stress, the depression is progressive (15 dps: - 51.0% control; 30 dps: - 61.0% control).

Under pH-stress the depression is conservatory (15 dps: - 68.6% control; 30 dps: - 62.4% control).

Under combinational stress, the depression is progressive as in the case of Cd-stress regime (15 dps: - 56.0% control, 30 dps: - 89.0% control).

The activity level of malate dehydrogenase in the gill tissue of O. senex senex under the different stressant regimes presents a picture of general depression (Table 5.32; Fig. 5.32; Statistical evaluation: Table 5.32a).

Under the Cd-regime, the depressory change is progressive (- 13.0% control, 15 dps; - 22.0% control, 30 dps).

Under pH-regime, the shorter stress-duration shows a very small negative change (- 3.7% control, 15 dps); longer stress-duration shows a positive change (+ 13.3% control, 30 dps) which too is not considerable.

Under the combinational regime in both stress-duration, an equal quantum of depression is evident (- 74.5% control).

The positive modulation i.e.
trend of elevation in the
activity levels of AChE,

V 4 COMMENT

ATPase system and both the
aminotransferases and a general trend of depression in
the activity levels of succinate, malate and lactate
dehydrogenase in the tissues of O. senex senex under
Cd -- and pH -- duresses show broad agreement with the
available literature (Roufogalis and Quist, 1972;
Hinton et al., 1973; Southard et al., 1974; Nitisewojo,
1977; Tucker, 1979; Sastry and Sharma, 1980b; Khangarot,
1981; Teagarden et al., 1981; Judith, 1982 and Srikanth,
1985).

TABLE 5.1: Effect of individual and combined in vivo stress of Cd & pH on AChE activity levels in O. senex senex.

(Values, expressed as μ moles of ACh metabolized/mg protein/h, are mean \pm SD of 6 determinations).

Control : 3.25 ± 0.09

Stress	15 d	Change % Control	30 d	Change % Control
Cd	3.60 ± 0.10	+ 10.8	3.81 ± 0.07	+ 17.2
pH	2.34 ± 0.52	- 28.0	4.30 ± 0.98	+ 32.3
Combined (Comb)	3.40 ± 0.11	+ 4.6	9.00 ± 0.13	+ 177

TABLE 5.1a: Comparison of means of AChE activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.1.

F = 403

CD = 0.712

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	S	NS	NS	S	S
Cd 15	-	S	NS	NS	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	NS	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.2: Effect of individual and combined in vivo stress of Cd & pH on Total ATPase activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.254 ± 0.01

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.285 ± 0.02	+ 12.2	0.183 ± 0.01	- 28.0
pH	0.350 ± 0.03	+ 37.8	0.185 ± 0.02	- 27.2
Combined (Comb)	0.263 ± 0.02	+ 3.5	1.301 ± 0.04	+ 412

TABLE 5.2a: Comparison of means of Total ATPase activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.2.

F = 3849

CD = 0.027

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S: Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.3: Effect of individual and combined in vivo stress of Cd & pH on Mg^{2+} ATPase activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of determinations).

Control : 0.133 ± 0.006

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.264 ± 0.02	+ 98.5	0.122 ± 0.01	- 8.3
pH	0.330 ± 0.02	+ 148	0.126 ± 0.01	- 5.3
Combined (Comb)	0.260 ± 0.02	+ 95.5	0.614 ± 0.03	+ 362

TABLE 5.3a: Comparison of means of Mg^{2+} ATPase activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.3.

F = 120

CD: 0.082

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	NS	NS	S
Cd 15	-	NS	NS	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.4: Effect of individual and combined in vivo stress of Cd & pH on AAT activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.300 ± 0.015

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.305 ± 0.02	+ 2.0	0.311 ± 0.02	+ 4.0
pH	0.031 ± 0.01	- 89.7	1.200 ± 0.12	+ 300
Combined (Comb)	0.520 ± 0.03	+ 73.3	1.204 ± 0.03	+ 301

TABLE 5.4a: Comparison of means of AAT activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.4.

F = 1666.5

CD = 0.052

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	S	S	NS	S	S
Cd 15	-	S	S	NS	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.5: Effect of individual and combined in vivo stress of Cd & pH on ALAT activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.200 ± 0.01

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.173 ± 0.014	- 13.5	0.140 ± 0.03	- 30.0
pH	0.063 ± 0.01	- 68.5	1.006 ± 0.04	+ 403
Combined (Comb)	0.167 ± 0.04	- 16.5	0.900 ± 0.02	+ 350

TABLE 5.5a: Comparison of means of ALAT activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.5.

F = 2757.14

CD = 0.031

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	S	S	S	S	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS: Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.6: Effect of individual and combined in vivo stress of Cd & pH on GDH activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.260 ± 0.03

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.065 ± 0.01	- 75.0	0.085 ± 0.01	- 61.3
pH	0.114 ± 0.012	- 56.0	0.142 ± 0.02	- 45.4
Combined (Comb)	0.240 ± 0.02	- 17.7	0.300 ± 0.04	+ 15.4

TABLE 5.6a: Comparison of means of GDH activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.6.

F = 425

CD = 0.028

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	S	NS
Cd 15	-	S	S	NS	S	S
pH 15	-	-	S	NS	NS	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.7: Effect of individual and combined in vivo stress of Cd & pH on SDH activity levels of CTGM in O. senex senex.

(Values, expressed as u moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.471 ± 0.04

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.180 ± 0.02	- 61.8	0.220 ± 0.014	- 53.3
pH	0.241 ± 0.016	- 48.8	0.120 ± 0.03	- 74.5
Combined (Comb)	0.380 ± 0.02	- 19.4	0.220 ± 0.03	- 53.3

TABLE 5.7a: Comparison of means of SDH activity levels in O. senex senex with reference to stress conditions presented in Table 5.7.

F = 142.25

CD = 0.074

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	S	NS	NS	NS
pH 15	-	-	S	NS	S	NS
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	NS
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.8: Effect of individual and combined in vivo stress of Cd & pH on LDH activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.262 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.121 ± 0.013	- 53.8	0.102 ± 0.01	- 61.0
pH	0.054 ± 0.02	- 79.4	0.150 ± 0.05	- 42.8
Combined (Comb)	0.074 ± 0.01	- 71.8	0.500 ± 0.05	+ 91.0

TABLE 5.8a: Comparison of means of LDH activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.8.

F = 361

CD = 0.037

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level, NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.9: Effect of individual and combined in vivo stress of Cd & pH on MDH activity levels of CTGM in O. senex senex.

(Values, expressed as μ moles for formazan formed/mg protein/h are mean \pm S.D. of 6 determinations).

Control : 0.127 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.070 ± 0.01	- 45.0	0.141 ± 0.01	+ 11.0
pH	0.100 ± 0.013	- 21.3	0.132 ± 0.04	+ 4.0
Combined (Comb)	0.075 ± 0.01	- 41.0	0.340 ± 0.044	+ 167

TABLE 5.9a: Comparison of means of MDH activity levels of CTGM in O. senex senex with reference to stress conditions presented in Table 5.9.

F = 295

CD = 0.028

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	NS	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.10: Effect of individual and combined in vivo stress of Cd & pH on AChE activity levels of M in O. senex senex.

(Values, expressed as μ moles of ACh metabolized/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 36.0 ± 3.50

Stress	15 d	Change % Control	30 d	Change % Control
Cd	73.0 ± 9.00	+ 102	102 ± 2.00	+ 183
pH	63.0 ± 1.50	+ 74.4	26.1 ± 6.50	- 27.5
Combined (Comb)	37.2 ± 8.41	+ 3.20	74.6 ± 9.40	+ 107

TABLE 5.10a: Comparison of means of AChE activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.10.

F = 661

CD = 7.69

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.11: Effect of individual and combined in vivo stress on Cd & pH on Total ATPase activity levels of M in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 1.66 ± 0.05

Stress	15 d	Change % Control	30 d	Change % Control
Cd	2.73 ± 0.45	+ 64.5	2.10 ± 0.10	+ 26.5
pH	3.00 ± 0.24	+ 81.0	1.80 ± 0.24	+ 8.4
Combined (Comb)	1.74 ± 0.20	+ 5.0	4.40 ± 0.16	+ 165

TABLE 5.11a: Comparison of means of Total ATPase activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.11.

F = 871

CD = 0.277

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	NS	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	NS	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.12: Effect of individual and combined in viv stress of Cd & pH on Mg^{2+} ATPase activity levels of M in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.535 + 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.770 \pm 0.06	+ 44.0	0.520 \pm 0.02	- 2.8
pH	1.300 \pm 0.09	+ 143	1.100 \pm 0.13	+ 106
Combined (Comb)	1.200 \pm 0.07	+ 124	1.530 \pm 0.03	+ 186

TABLE 5.12a: Comparison of means of Mg^{2+} ATPase activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.12.

F = 154

CD = 0.082

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	NS	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.13: Effect of individual and combined in vivo stress of Cd & pH on AAT activity levels of M in O. senex senex.

(Values, expressed as μ moles of pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.400 ± 0.03

Stress	15 d	Change% Control	30 d	Change % Control
Cd	0.700 ± 0.04	+ 75.0	0.630 ± 0.08	+ 57.5
pH	0.520 ± 0.05	+ 30.0	1.730 ± 0.15	+ 333
Combined (Comb)	1.000 ± 0.042	+ 150	1.500 ± 0.05	+ 275

TABLE 5.13a: Comparison of means of AAT activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.13.

F = 1872

CD = 0.074

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.14: Effect of individual and combined in vivo stress of Cd & pH on ALAT activity levels of M in O. senex senex.

(Values, expressed as μ moles of pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.621 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.322 ± 0.20	+ 113	0.672 ± 0.05	+ 8.2
pH	0.664 ± 0.06	+ 7.6	1.500 ± 0.12	+ 142
Combined (Comb)	1.300 ± 0.30	+ 109	2.320 ± 0.20	+ 274

TABLE 5.14a: Comparison of means of ALAT activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.14.

F = 493

CD = 0.185

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	S	S
Cd 15	-	S	NS	S	NS	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.15: Effect of individual and combined in vivo stress of Cd & pH on GDH activity levels of M in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.360 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.104 ± 0.01	- 71.2	0.200 ± 0.05	- 44.5
pH	0.182 ± 0.03	- 49.5	0.071 ± 0.02	- 80.0
Combined (Comb)	0.300 ± 0.05	- 16.7	0.350 ± 0.03	- 2.8

TABLE 5.15a: Comparison of means of GDH activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.15.

F = 345

CD = 0.04

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.16: Effect of individual and Combined in vivo stress of Cd & pH on SDH activity levels of M in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.424 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.108 ± 0.02	- 74.5	0.160 ± 0.03	- 62.3
pH	0.200 ± 0.03	- 52.8	0.053 ± 0.02	- 87.5
Combined (Comb)	0.305 ± 0.04	- 28.0	0.620 ± 0.05	+ 46.2

TABLE 5.16a: Comparison of means of SDH activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.16.

F = 660

CD = 0.038

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level, NS: Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.17: Effect of individual and combined in vivo stress of Cd & pH on LDH activity levels of M in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.252 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.119 ± 0.02	- 52.8	0.200 ± 0.05	- 20.0
pH	0.175 ± 0.05	- 30.0	0.020 ± 0.01	- 92.0
Combined (Comb)	0.240 ± 0.02	- 4.8	0.110 ± 0.03	- 56.4

TABLE 5.17a: Comparison of means of LDH activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.17.

F = 174

CD = 0.04

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	S	S
Cd 15	-	S	S	S	S	NS
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.18: Effect of individual and combined in vivo stress of Cd & pH on MDH activity levels of M in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.151 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.140 ± 0.02	- 7.0	0.110 ± 0.014	- 27.0
pH	0.161 ± 0.014	+ 6.6	0.070 ± 0.02	- 53.7
Combined (Comb)	0.205 ± 0.02	+ 35.8	0.140 ± 0.02	- 7.0

TABLE 5.18a: Comparison of means of MDH activity levels of M in O. senex senex with reference to stress conditions presented in Table 5.18.

F = 338

CD = 0.023

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	S	S	S	NS
Cd 15	-	NS	S	S	S	NS
pH 15	-	-	S	S	S	NS
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	.	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.19: Effect of individual and combined in vivo stress of Cd & pH on Total ATPase activity levels of HP in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 1.62 ± 0.05

Stress	15 d	Change % Control	30 d	Change % Control
Cd	3.14 ± 0.20	+ 94.0	1.04 ± 0.07	- 35.8
pH	1.70 ± 0.13	+ 5.0	0.80 ± 0.053	- 50.0
Combined (Comb)	1.78 ± 0.05	+ 9.9	4.01 ± 0.012	+ 148.

TABLE 5.19a: Comparison of means of Total ATPase activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.19.

F = 3548

CD = 0.111

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.20: Effect of individual and combined in vivo stress of Cd & pH on Mg^{2+} ATPase activity levels of HP in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.717 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.53 ± 0.10	+ 113	0.563 ± 0.02	- 21.5
pH	1.20 ± 0.04	+ 67.4	0.453 ± 0.02	- 36.8
Combined (Comb)	1.042 ± 0.03	+ 45.3	1.30 ± 0.05	+ 81.3

TABLE 5.20a: Comparison of means of Mg^{2+} ATPase activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.20.

F = 2771

CD = 0.064

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.21: Effect of individual and combined in vivo stress of Cd & pH on AAT activity levels of HP in O. senex senex.

(Values, expressed as μ moles of Pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.863 ± 0.03

Stress	15 d	Change % Control	30 d	Change % Control
Cd	4.00 ± 0.80	+ 364	1.03 ± 0.13	+ 19.4
pH	0.913 ± 0.07	+ 5.8	3.00 ± 0.16	+ 248
Combined (Comb)	1.43 ± 0.12	+ 65.7	3.00 ± 0.05	+ 248

TABLE 5.21a: Comparison of means of AAT activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.21.

F = 371.1

CD = 0.37

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.22: Effect of individual and combined in vivo stress of Cd & pH on ALAT activity levels of HP in O. senex senex.

(Values, expressed as μ moles of pyruvate formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.565 ± 0.05

Stress	15 d	Change % Control	30 d	Change % Control

Cd	2.50	+ 343	0.66	+ 17.0
	± 0.40		± 0.09	
pH	0.50	- 11.5	2.80	+ 396
	± 0.07		± 0.10	
Combined (Comb)	1.00	+ 77.0	2.20	+ 290
	± 0.10		± 0.23	

TABLE 5.22a: Comparison of means of ALAT activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.22.

F = 679

CD = 0.205

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	NS	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	.	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.23: Effect of individual and combined in vivo stress of Cd & pH on **GDH** activity levels of HP in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.134 ± 0.01

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.524 ± 0.09	+ 291	0.207 ± 0.04	+ 54.5
pH	0.107 ± 0.03	- 20.0	0.100 ± 0.01	- 25.4
Combined (Comb)	0.118 ± 0.02	- 12.0	0.233 ± 0.05	+ 74.0

TABLE 5.23a Comparison of means of GDH activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.23.

F = 221

CD = 0.052

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	NS	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	S
Comb 15	-	-	-	S	NS	S
Cd 30	-	-	-	-	S	NS
pH 30	-	.	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.24: Effect of individual and combined in vivo stress of Cd & pH on SDH activity levels of HP in O. senexssenex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.275 ± 0.01

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.530 ± 0.50	+ 456	0.666 ± 0.06	+ 142
pH	0.333 ± 0.08	+ 20.0	0.427 ± 0.09	+ 55.3
Combined (Comb)	0.266 ± 0.05	- 3.3	0.300 ± 0.13	+ 9.1

TABLE 5.24a: Comparison of means of SDH activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.24.

F = 82.5

CD 0.234

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	NS	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	.	-	-	-	NS

S : Significant at 5% level, NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.25: Effect of individual and combined in vivo stress of Cd & pH on LDH activity levels of HP in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.121 ± 0.012

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.578 ± 0.08	+ 378	0.240 ± 0.05	+ 98.3
pH	0.130 ± 0.03	+ 7.4	0.044 ± 0.014	- 63.6
Combined (Comb)	0.130 ± 0.022	+ 7.4	0.200 ± 0.024	+ 65.3

TABLE 5.25a: Comparison of means of LDH activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.25.

F = 283.6

CD = 0.052

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	NS
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.26: Effect of individual and combined in vivo stress of Cd & pH on MDH activity levels of HP in O. senex senex

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.250 ± 0.03

Stress	15 d	Change % Control	30 d	Change % Control
		- - - - -	- - - - -	- - - - -
Cd	0.650 ± 0.09	+ 160	0.334 ± 0.09	+ 33.6
pH	0.143 ± 0.05	- 42.8	0.120 ± 0.04	+ 52.0
Combined (Comb)	0.102 ± 0.01	- 59.2	0.875 ± 0.05	+ 250

TABLE 5.26a: Comparison of means of MDH activity levels of HP in O. senex senex with reference to stress conditions presented in Table 5.26.

F = 400.3

CD : 0.069

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	S
Comb 15	-	-	-	S	NS	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.27: Effect of individual and combined in vivo stress of Cd & pH on Total ATPase activity levels of G in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.922 ± 0.03

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.550 ± 0.05	- 40.0	0.650 ± 0.03	- 29.5
pH	1.124 ± 0.05	+ 22.0	0.400 ± 0.04	- 56.6
Combined (Comb)	1.000 ± 0.14	+ 8.5	1.650 ± 0.05	+ 79.0

TABLE 5.27a: Comparison of means of Total ATPase activity levels of G in O. senex senex with reference to stress conditions presented in Table 5.27.

F = 1524.7

CD : 0.077

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.28: Effect of individual and combined in vivo stress of Cd & pH on Mg^{2+} ATPase activity levels of G in O. senex senex.

(Values, expressed as μ moles of Pi formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.600 \pm 0.023

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.423 \pm 0.013	- 29.5	0.298 \pm 0.02	- 50.0
pH	0.730 \pm 0.03	- 22.0	0.325 \pm 0.032	- 45.2
Combined (Comb)	0.611 \pm 0.04	+ 2.0	0.725 \pm 0.033	+ 21.0

TABLE 5.28a: Comparison of means of Mg^{2+} ATPase activity levels of G in O. senex senex with reference to stress conditions presented in Table 5.28.

F = 2514

CD = 0.034

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	NS	S	S	S
Cd 15	-	S	S	S	S	S
pH 15	-	-	S	S	S	NS
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.29: Effect of individual and combined in vivo stress of Cd & pH on GDH activity levels of G in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.625 ± 0.03

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.302 ± 0.10	- 51.7	0.250 ± 0.11	- 60.0
pH	0.302 ± 0.04	- 51.7	0.610 ± 0.07	- 2.4
Combined (Comb)	0.362 ± 0.06	- 42.0	0.023 ± 0.01	- 96.3

TABLE 5.29a: Comparison of means of GDH activity levels of G in Q. senex senex with reference to stress conditions presented in Table 5.29.

F = 186.6

CD = 0.082

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	NS	S
Cd 15	-	NS	NS	NS	S	S
pH 15	-	-	NS	NS	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S: Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.30: Effect of individual and combined in vivo stress of Cd & pH on SDH activity levels of G in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.983 ± 0.02

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.513 ± 0.04	- 47.2	0.341 ± 0.17	- 65.7
pH	1.002 ± 0.09	+ 2.0	0.665 ± 0.07	- 32.4
Combined (Comb)	0.424 ± 0.09	- 57.0	0.150 ± 0.015	- 85.0

TABLE 5.30a: Comparison of means of SDH activity levels of G in O. senex senex with reference to stress conditions presented in Table 5.30.

F = 284.45

CD = 0.104

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	S	S	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	NS	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level, NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.31: Effect of individual and combined in vivo stress of Cd & pH on LDH activity levels of G in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.274 ± 0.04

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.134 ± 0.06	- 51.0	0.107 ± 0.02	- 61.0
pH	0.086 ± 0.007	- 68.6	0.103 ± 0.02	- 62.4
Combined (Comb)	0.120 ± 0.013	- 56.0	0.030 ± 0.008	- 89.0

TABLE 5.31a: Comparison of means of LDH activity levels of G in O. senex senex with reference to stress conditions presented in Table 5.31.

F = 367.13

CD = 0.023

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	S	NS	S	S	S
pH 15	-	-	S	NS	NS	S
Comb 15	-	-	-	NS	NS	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant

CD value was calculated according to the formula given in Table 3.1a.

TABLE 5.32: Effect of individual and combined in vivo stress of Cd & pH on MDH activity levels of G in O. senex senex.

(Values, expressed as μ moles of formazan formed/mg protein/h, are mean \pm S.D. of 6 determinations).

Control : 0.353 ± 0.07

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.307 ± 0.02	- 13.0	0.277 ± 0.02	- 22.9
pH	0.340 ± 0.07	- 3.7	0.400 ± 0.05	+ 13.2
Combined (Comb)	0.090 ± 0.009	- 74.5	0.090 ± 0.009	- 74.5

TABLE 5.32a: Comparison of means of MDH activity levels of G in O. senex senex with reference to stress conditions presented in Table 5.32.

F = 268

CD = 0.052

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	S	S	NS	S
Cd 15	-	NS	S	NS	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level, NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

Fig. 5.1: Percent change of AChE activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.2: Percent change of Total ATPase activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.3: Percent change of Mg^{2+} ATPase activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

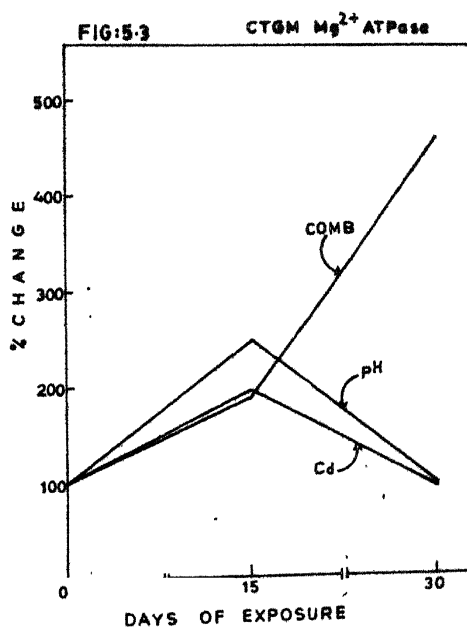
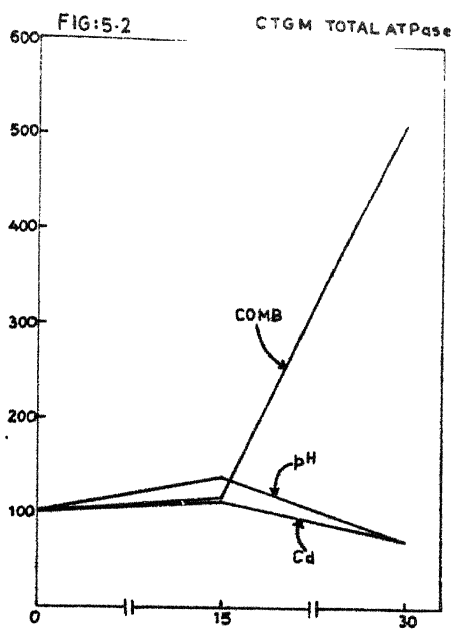
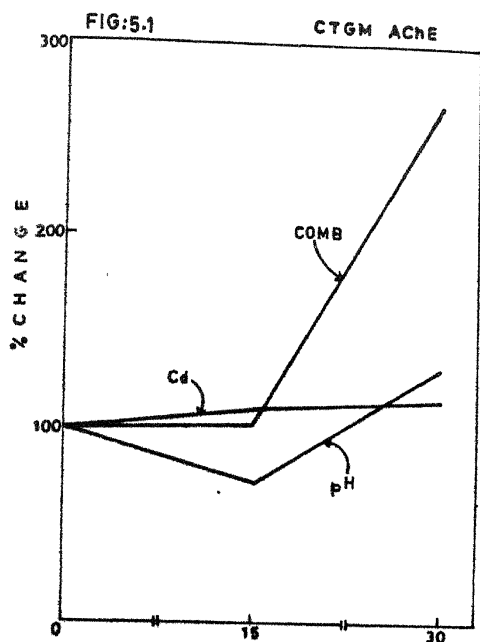


Fig. 5.4: Percent change of AAT activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.5: Percent change of AlAT activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.6: Percent change of GDH activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.7: Percent change of SDH activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

FIG:5.4

CTGM AAT

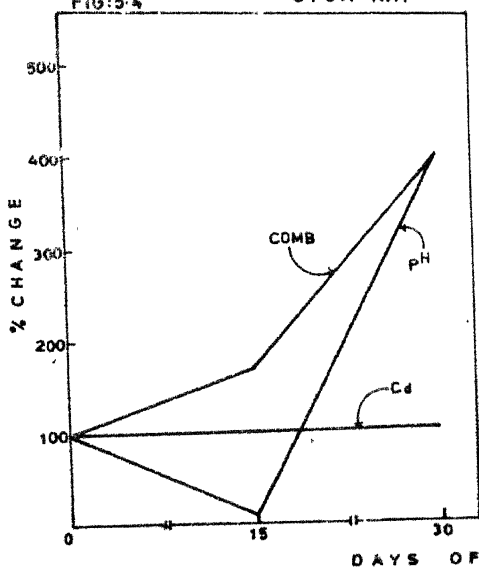


FIG:5.5

CTGM AIAT

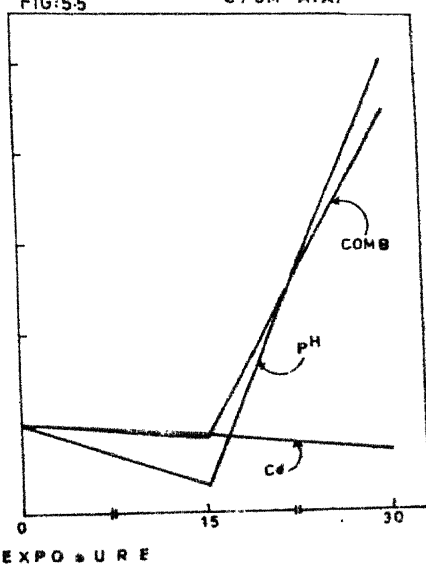


FIG:5.6

CTGM GDH

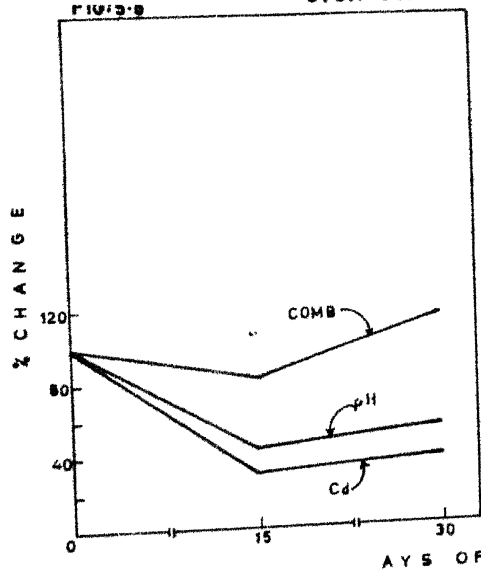


FIG:5.7

CTGM SDH

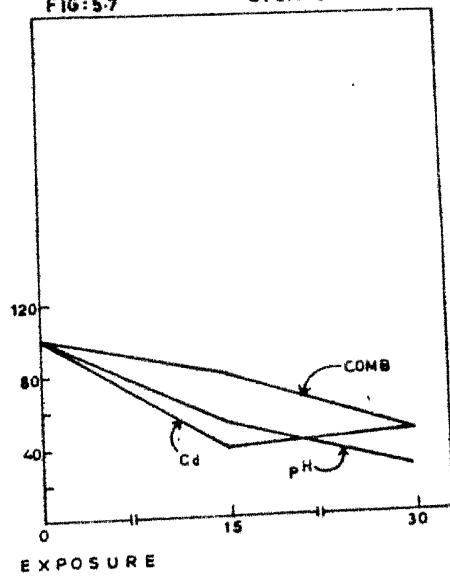


Fig. 5.8: Percent change of LDH activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.9: Percent change of MDH activity level in cephalothoracic ganglionic mass (CTGM) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.10: Percent change of AChE activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

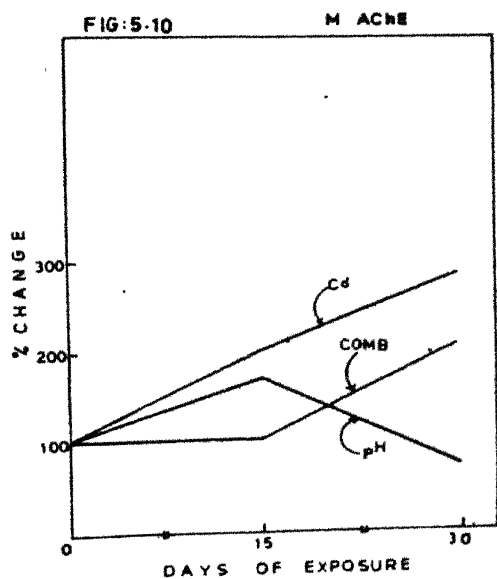
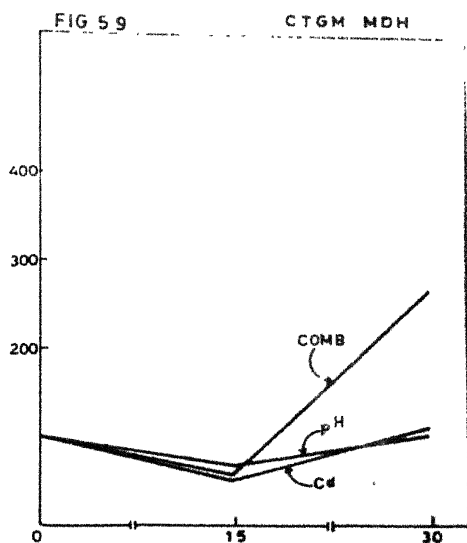
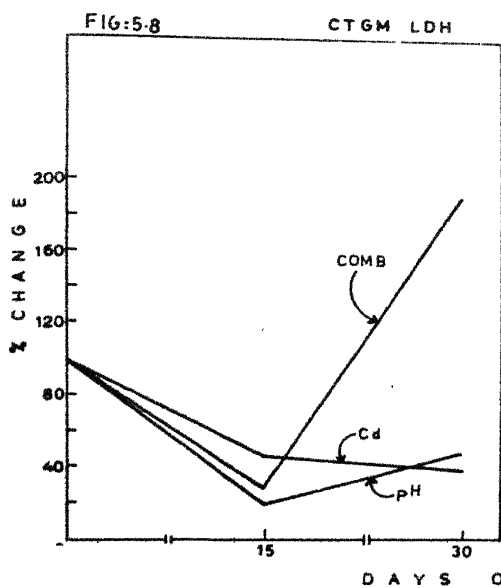
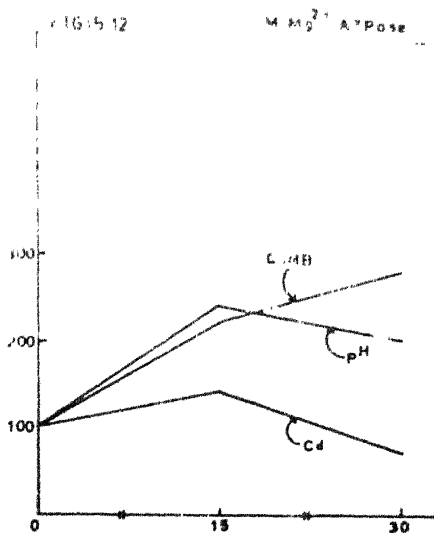
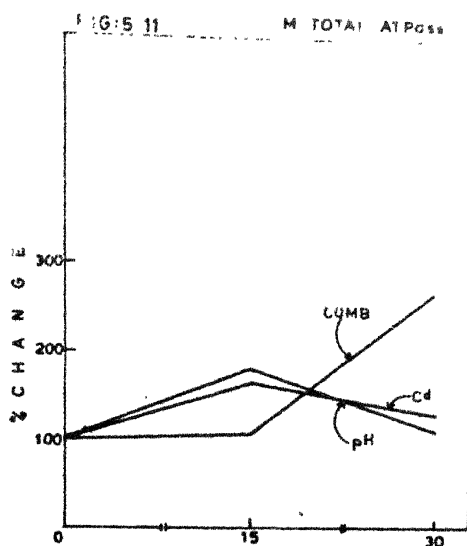


Fig. 5.11: Percent change of Total ATPase activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

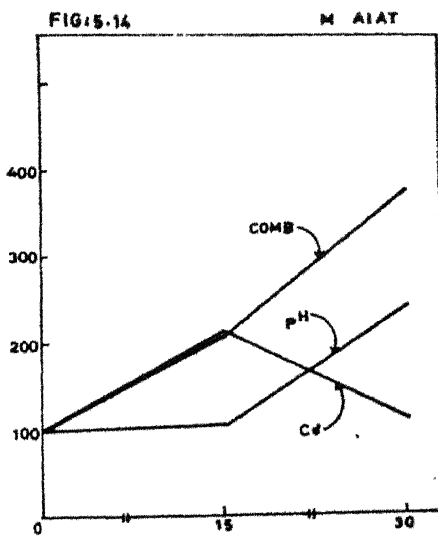
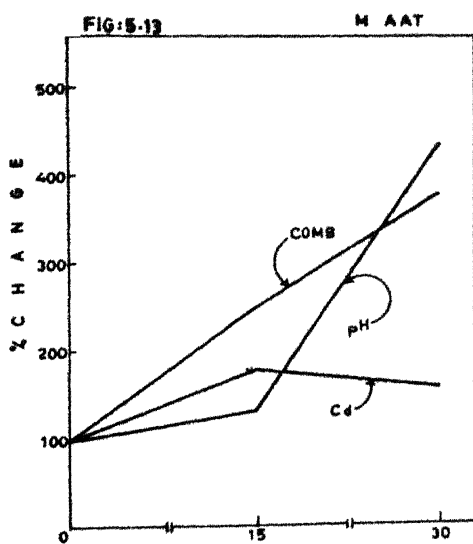
Fig. 5.12: Percent change of Mg^{2+} ATPase activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.13: Percent change of AAT activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.14: Percent change of AlAT activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.



DAYS OF EXPOSURE



DAYS OF EXPOSURE

- Fig. 5.15: Percent change of GDH activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 5.16: Percent change of SDH activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 5.17: Percent change of LDH activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.
- Fig. 5.18: Percent change of MDH activity level in muscle (M) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30d sublethal exposure periods.

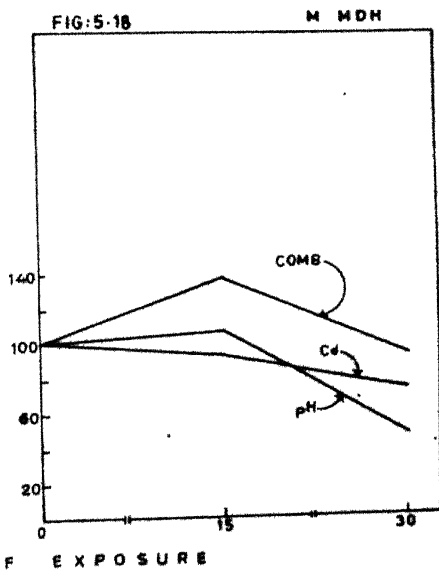
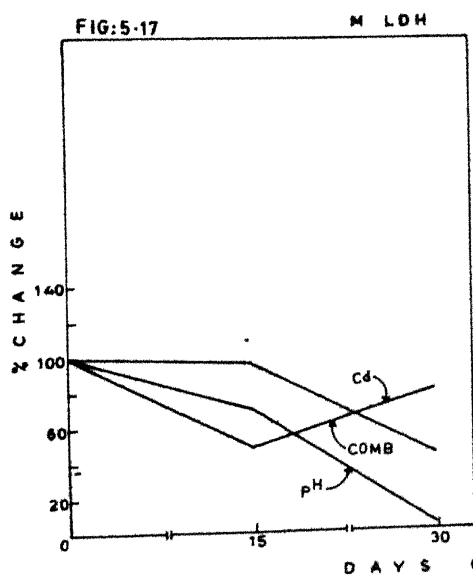
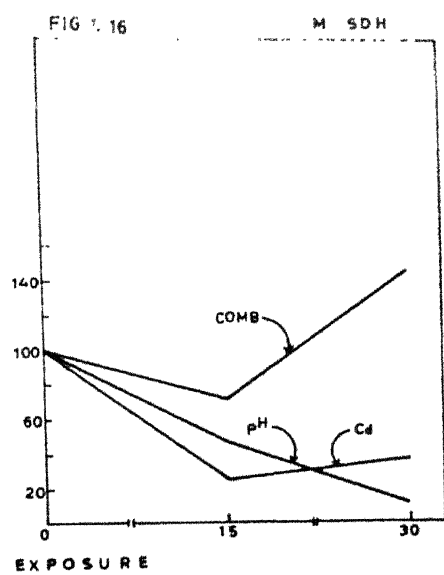
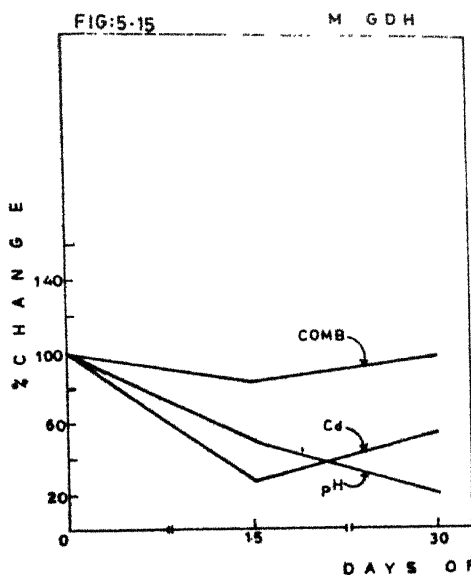


Fig. 5.19: Percent change of Total ATPase activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.20: Percent change of Mg^{2+} ATPase activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.21: Percent change of AAT activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.22: Percent change of AlAT activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

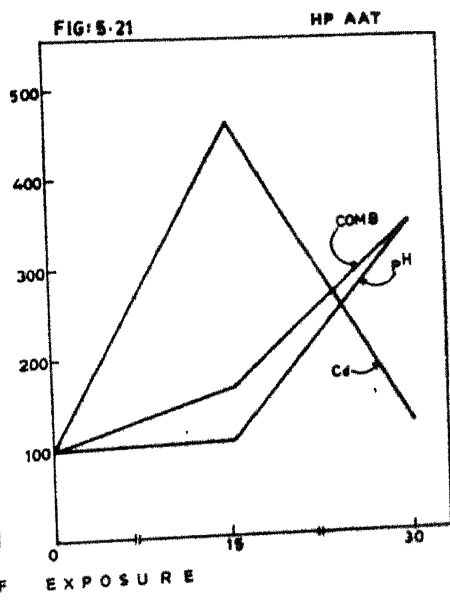
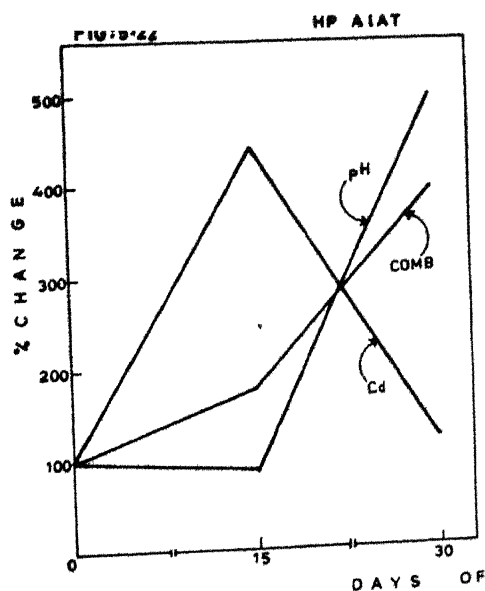
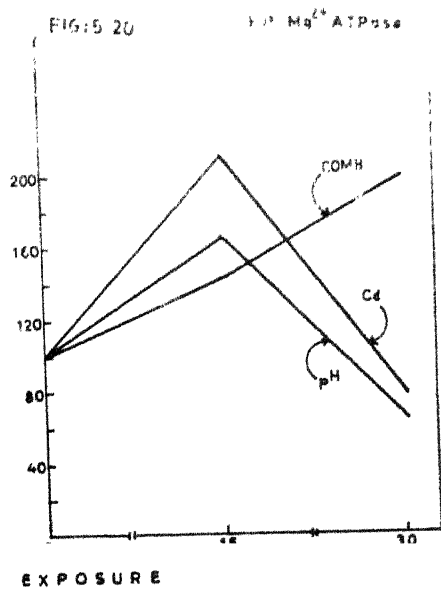
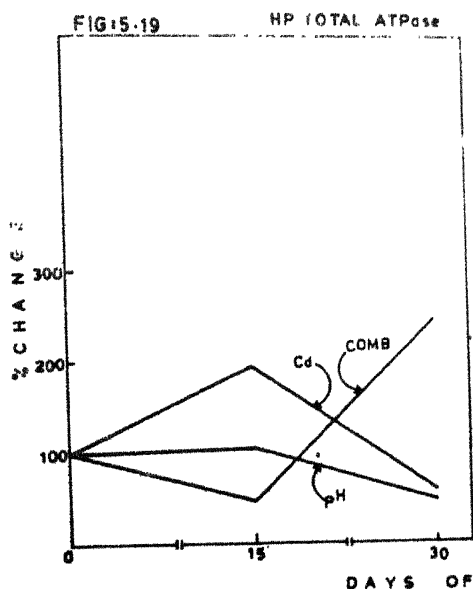


Fig. 5.23: Percent change of GDH activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.24: Percent change of SDH activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.25. Percent change of LDH activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.26: Percent change of MDH activity level in hepatopancreas (HP) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

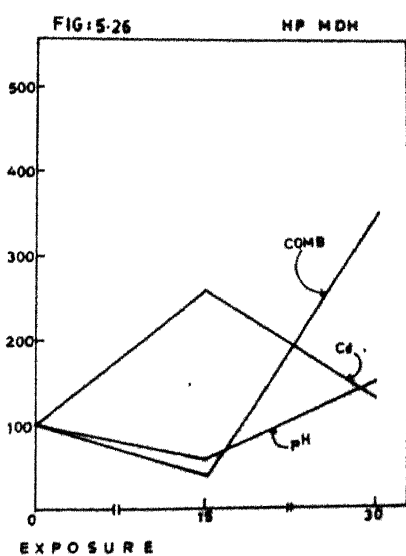
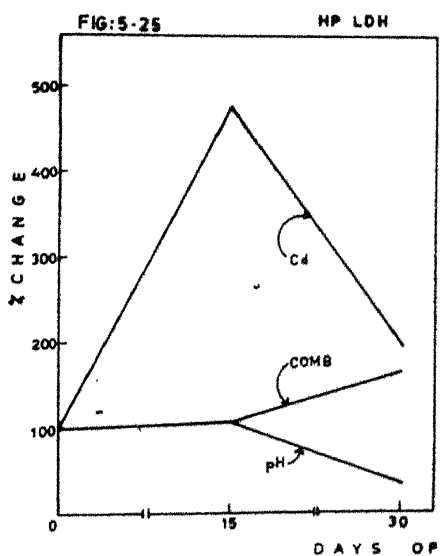
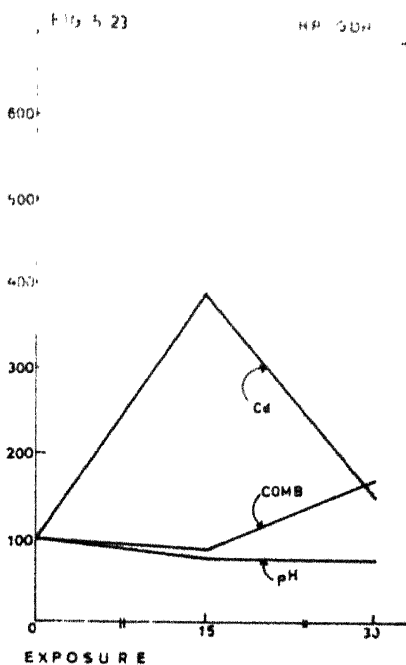
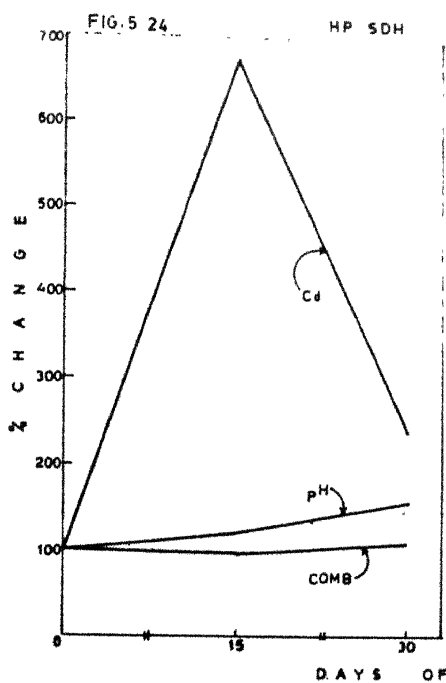
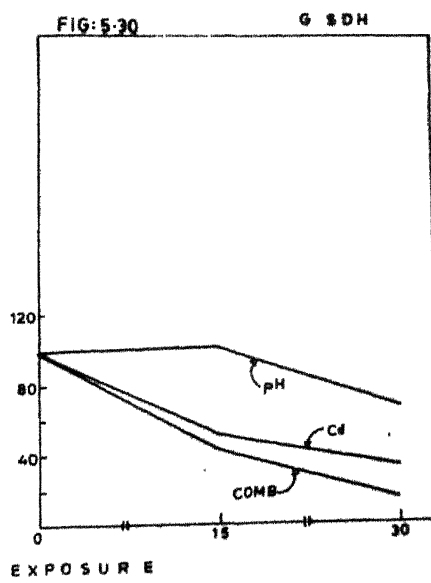
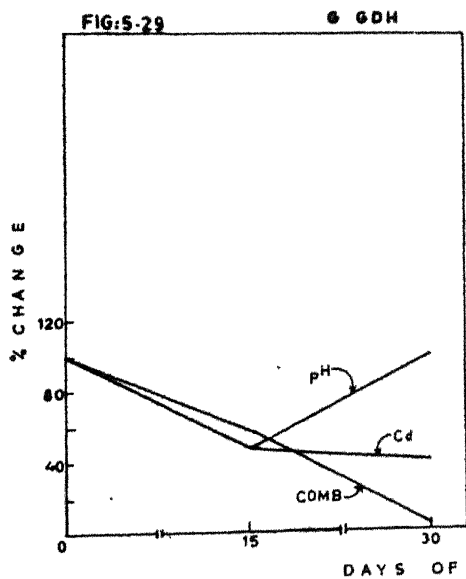
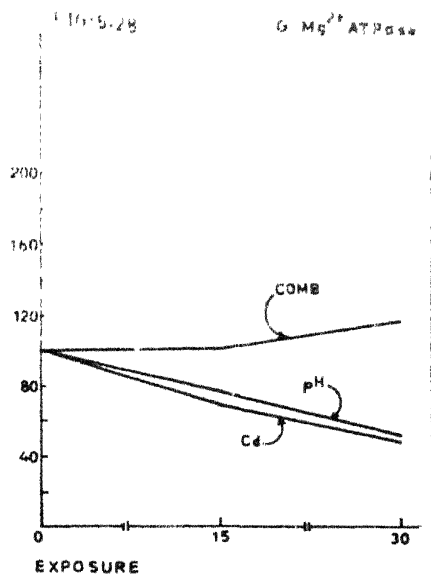
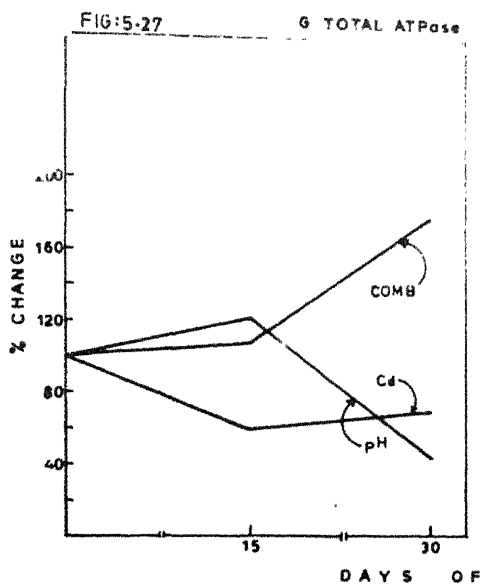


Fig. 5.27: Percent change of Total ATPase activity level in gill (G) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.28: Percent change of Mg^{2+} ATPase activity level in gill (G) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.29: Percent change of GDH activity level in gill (G) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.

Fig. 5.30: Percent change of SDH activity level in gill (G) of Cd-, pH- and Combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal exposure periods.



CHAPTER VI

HISTOGRAVIMETRY AND TISSUE HYDRATION PROFILES

The stressants exert influence on the levels of chemical constituents of the tissues. This point has been made out statistically in the present organism (O. senex senex) under Cd- and pH-duresses. Examination of these chemical profiles no doubt forms an important elucidational methodology in toxicology. However, examination of the gravimetric statuses of the sub-organismal components (Viz., tissues) under the stressant-duress may give an additional interpretational dimension. This gravimetric approach has earlier been employed for the study of stresses like aestival dehydration (Chandrasekharam, 1977) and hyperosmotic salinity-adaptation stress (Venkatareddy, 1976).

In this chapteral location, the data on the wet and dry weight statuses of the tissues in relation to the stressant-duress are in given for O. senex senex. Incidentally the levels of tissue hydration are also dealt with, which data flow by way of difference between wet and dry tissue weights.

VI.2 MATERIALS AND METHODS

VI.2.A HSI DETERMINATION have been given elsewhere

The procedural details regarding collection of animals and their in-laboratory maintenance

(Chapter II). Preparation of the different investigation groups also has been given earlier (Chapters II and III). Tissue isolation procedures are dealt with in an earlier chapteral location (Chapter IV).

Tissue gravimetry consists in isolating the tissue in totality, as far as practicable, and recording the weight of this tissue. This total tissue weight, expressed in somatic weight-specific terms, yields an index, named 'histo-somatic index' (HSI).

$$\text{HSI} = \frac{\text{Total tissue weight (g)}}{\text{Somatic weight (g)}} \times 100 \quad \dots\dots (1)$$

If the total wet tissue weight is expressed percent somatic weight, wet HSI is obtained. Similar computation with the total dry weight of the tissue yields dry HSI.

The stressed organisms, and control-groups are used for determination of the HSIs of the different tissues.

Thus HSI determination involves, recording of the somatic weight of an organism and recording of total individual tissue-weight after sacrifice of the organism.

Tissue like hepatopancreas, gill and gonad, can be isolated easily. But isolation of chelate leg muscle is fraught with problems; therefore, an indirect method is employed for the determination of the total weight of this tissue, as described by Venkatarreddy (1976) earlier. This method involves determination of weight of chelate legs, their alkaline-digestion (for dissolution of muscle mass) and determination of the muscle-free chelate-leg skeleta.

The chelate leg pair is scissored from the organism, and the weight is determined in analytical balance (Entire leg weight, EW). The skeletal enclosures of the legs are slit open to expose the muscles and the 'scissorable' mass is removed. This preparation is kept in small KOH-solution filled beakers overnight, to allow digestion of the muscle mass remaining attached to the skeletal interiors. Next day, the skeleta are removed, thoroughly washed in water and dried. The weight of this chelate leg skeleton is determined (skeletal weight, SW). The difference between these two weights gives the weight of chelate leg muscle (MW).

$$MW = (ES - SW) \quad \dots \quad (2)$$

The MW thus obtained may then be employed for computation of HSI of muscle tissue.

The tissues, after isolation, are blotted free of adhering body fluids and weighed to obtain wet tissue weight records. The tissues are dried in hot air oven at 65°C for 10 h and dry weights are recorded.

In the case of muscle, major mass obtained after 'chelotomy' (chelate leg slitting) is weighed, dried and weighed (for wet and dry weight recordings).

Finally, a correction is applied to the dry weight, of muscle, after assessing more accurately the wet weight of the chelate leg muscle by the indirect alkaline digestion method mentioned above.

VI 2.B RECORDING OF TISSUE HYDRATION

The recording of tissue hydration is incidental to determination of wet and dry weights of tissues. This important component of the tissues is expressed here in relation to somatic weight. Total tissue water (TTW) is obtained by way of difference between tissue wet weight (TWW) and tissue dry weight (TDW), the recording procedures of which are given above.

$$TTW = TWW - TDW \quad \dots \quad (3)$$

TTW expressed percent somatic weight (SW) yields an index, water-somatic index (WSI) of the tissue.

$$WSI = \frac{TTW}{SW} \times 100 \quad \dots \quad (4)$$

A more useful parameter which yields insight into the physiological state of the tissue is tissue-weight specific water content or tissue-weight specific hydration (WSH) of water content percentage.

$$WSH = \frac{TTW}{TWW} \times 100 \quad \dots \quad (5)$$

VI 2.C STATISTICAL ANALYSIS

The tissue gravimetric and hydration data obtained as detailed above are subjected to statistical evaluation using analysis of variance ('ANOVA') as the statistical analytical tool (Pillai and Sinha, 1968).

VI 3 RESULTS

The results of the histogravimetric investigation and studies of hydration levels in

6. senex senex, in relation to the diverse stressant regimes are given in tables 6.1 to 6.12. Tissue weight-specific hydration (WSH) data are given in tables 6.I to 6.IV.

Wet gravimetric data for
hepatopancreas of O. senex

VI 3.A HEPATOPANCREAS (HP) senex in relation to divers
stressantduresses are given
in table 6.1 (Fig. 6.1; Statistical Evaluation: Table
6.1a).

Under the different stressant regimes, the wet
tissue weight status of hepatopancreas shows a consis-
tent depressive modification.

Under Cd-regime, the pattern is conservatory
depression (- 54.4% control, 15 dps; - 27.6% control,
30 dps).

Under pH-regime too, a similar trend is evident
(- 43.3% control, 15 dps; - 38.8% control, 30 dps).

Under combinational regime, a reversed trend is
discernible (- 37.5% control, 15 dps; - 47.5% control,
30 dps) (Fig. 6.1).

Dry weight of hepatopancreas also is modified
in the negative (depression) direction under different
stressant duresses in O. senex senex (Table 6.2; Fig. 6.2;
Statistical evaluation: Table 6.2a).

Cd-induced change in dry tissue weight status
is conservatory depression (15 dps: - 56.3% control;
30 dps: - 17.0% control).

Under pH-stress the trend of progressive depression is evident (15 dps: - 41.5% control; 30 dps: - 46.0% control).

Under combinational regime also progressive depression trend is noted (15 dps: - 44.4% control; 30 dps: - 56.6% control).

Water-somatic indices (WSIs) for hepatopancreas undergo depressions consistently under the different stressant regimes, in O. senex senex (Table 6.3; Fig. 6.3; Statistical evaluation: Table 6.3a)

Under the individual stresses of Cd and pH, the WSI's show the trend of conservatory depression (Cd: 15 dps: - 53.2% control; 30 dps: - 34.9% control; pH: 15 dps: - 42.7% control; 30 dps: - 33.6% control).

Under the combinational regime, the trend of progressive depression is evident (15 dps: -32.8% control, 30 dps: - 47.5% control).

Hepatopancreatic tissue weight specific hydration (WSH) under the stressant duresses shows diverse deviations (Table 6.I). Under Cd-stress, the shorter stress-duration shows a small increase in this parameter (+ 2.8% control, 15 dps). Depressory change is found in the longer stress-duration (-10.1% control, 30 dps).

Under pH stress, the parameter shows progressive elevation profile (15 dps: + 1.2% control; 30 dps: + 8.6% control).

Under combinational regime, the shorter stress-duration shows a positive change (+ 7.6% control, 15 dps) while in the longer stress-duration, the parameter shows zero-percent change.

Wet gravimetric data for
chelate leg muscle of O. senex
VI 3.B CHELATE LEG
MUSCLE (M) senex under the different
stressant regimes are given in table 6.4 and figure 6.4
(Statistical evaluation: Table 6.4a).

The wet weight status of the tissue undergoes consistently positive change under the different stressant regimes.

Under Cd-regime, the pattern is of progressive elevation (15 dps: + 11.5% control; 30 dps: + 12.3% control).

Under pH-regime, the pattern is conservatory elevation (+ 16.3% control, 15 dps; + 10.5% control; 30 dps).

Under combinational regime also conservatory elevation pattern is found (+ 9.3% control, 15 dps; + 5.8% control, 30 dps).

Under the stressant regimes, the dry weight status of muscle shows a general trend of positive change (Table 6.5; Fig. 6.5; Statistical evaluation: Table 6.5a).

Under Cd-regime, a pattern of progressive elevation is discernible (+ 5.2% control, 15 dps; + 20.1% control, 30 dps).

Under pH-regime also a similar pattern is evident (+ 6.2% control, 15 dps; + 20.0% control, 30 dps).

Under combinational regime, a depression-elevation pattern is found (- 12.0% control, 15 dps; + 8.7% control, 30 dps).

The parameter of water somatic index of muscle of O. senex senex shows a consistent elevatory trend under the different stressant regimes (Table 6.6; Fig. 6.6; Statistical evaluation: Table 6.6a).

Under all the regimes, the pattern of conservatory elevation is noticeable (Cd-stress: + 18.3% control, 15 dps; + 4.2% control, 30 dps; pH-stress: +27.3% control, 15 dps; + 0.6% control, 30 dps; Combinational stress: + 32.1% control, 15 dps; + 21.6% control, 30 dps).

Tissue weight-specific hydration (WSH) data for muscle of O. senex senex under the different stressant regimes are given in table 6.II.

Under Cd-stress, a pattern of elevation-depression is evident (+ 6.0% control, 15 dps; - 7.4% control, 30 dps).

Under pH-stress also a similar pattern is discernible (+ 9.3% control, 15 dps; - 9.1% control, 30 dps). Under combinational regime a pattern of conservatory elevation is evident (+ 20.8% control, 15 dps; + 14.8% control, 30 dps).

VI 3.C GILL (G) The wet branchial histosomatic indices of O. senex under the stressant regimes are given in table 6.7, figure 6.7 (Statistical evaluation: Table 6.7a).

Under Cd-regime, the shorter stress-duration shows a small, non-significant depression of branchial HSI (- 6.0% control, 15 dps). At the longer stress-duration, there is a notable elevation of the index (+ 74.8% control, 30 dps).

Under pH-stress, a pattern of progressive elevation is evident (15 dps: + 8.4% control; 30 dps: + 87.8% control).

Under the combinational regime, both durations reveal statistically non-significant variations. In the shorter stress-duration a 9.1% elevation is found

while in the longer stress-duration a very small negative change is found (- 1.1% control, 30 dps).

The dry histosomatic index data for gill tissue of O. senex senex under the stressant-regimes are given in table 6.8 and figure 6.8 (Statistical evaluation: Table 6.8a).

Under the regimes, a trend of general elevation of the HSI status of the tissue is evident.

Under Cd-stress, a pattern of progressive elevation is discernible (+ 5.6% control, 15 dps; + 35.0% control, 30 dps). Under pH-stress, the shorter stress-duration shows a small positive change (+ 11.1% control 15 dps) and the longer stress-duration shows a very small depression (- 3.9% control, 30 dps).

Under the combinational regime, a pattern of conservatory elevation is evident (15 dps: + 28.3% control, 30 dps: + 12.8% control).

The water-somatic index data for the gill tissue in O. senex senex reveal a general trend of elevation, under the diverse stress regimes (Table 6.9; Fig. 6.9; Statistical evaluation: Table 6.9a)

Under Cd-stress, the pattern of depression-elevation is found (15 dps: - 7.7% control; 30 dps: +79.4% control).

Under pH-stress, the pattern of progressive elevation is found (15 dps: + 8.8% control; 30 dps: + 92.0% control).

Under the combinational regime, small, statistically non-significant changes are noted in both durations (+ 6.4% control, 15 dps; - 3.1% control, 30 dps).

The weight-specific hydration data for gill tissue of O. senex senex are given in table 6.III.

Under Cd-stress, the pattern of depression-elevation is evident (- 1.7% control, 15 dps; + 2.6% control, 30 dps).

Under pH-regime, the shorter stress-duration shows a very small elevation of WSH (+ 0.3% control, 15 dps) while the longer stress-duration shows an elevation (+ 5.6% control, 30 dps).

Under the combinational regime, both durations show a small, negative changes of WSH (- 2.4% control, 15 dps; - 2.1% control, 30 dps).

VI 3.D OVARY (O)

The ovarian wet histosomatic index data in O. senex senex

(Table 6.10; Fig. 6.10; Statistical evaluation: Table 6.10a) show a general elevatory trend.

Under Cd-regime the changes, which are remarkably high, show the pattern of conservatory elevation (+ 362% control, 15 dps; + 143% control, 30 dps).

Under pH-regime the pattern is progressive elevation (+ 48.0% control, 15 dps; + 135% control, 30 dps).

Under the combinational regime the pattern of elevation-depression is evident (+ 66.0% control, 15 dps; - 23.5% control, 30 dps).

The dry gravimetric status of ovary in O. senex senex varies generally on elevation side, under various stressant regimes (Table 6.11; Fig. 6.11; Statistical evaluation: 6.11a).

Under Cd-stress, the remarkably high elevations noted show the pattern of conservation (+ 374% control, 15 dps; + 197% control, 30 dps).

Under pH-regime, the pattern of progressive elevation is observable (+ 66.7% control, 15 dps; + 78.0% control, 30 dps).

Under the combinational regime, the pattern of elevation-depression is evident (15 dps: + 107% control; 30 dps: - 46.7% control).

The water-somatic index of ovary in O. senex under the different stressant regimes shows a general trend of elevation (Table 6.12; Fig. 6.12; Statistical evaluation: Table 6.12a).

Under Cd-regime, the pattern of conservatory elevation is evident (+ 352% control, 15 dps; + 107% control, 30 dps).

Under pH-regime, the pattern of progressive elevation is evident (+ 41.2% control, 15 dps; + 171% control, 30 dps).

Under the combinational regime, the pattern of elevation-depression is evident (+ 41.2% control, 15 dps; - 5.9% control, 30 dps).

The ovarian tissue weight-specific hydration (WSH) levels in O. senex, under the different stressant regimes are given in Table 6.IV.

Under Cd-regime, the change is of the progressive elevation pattern (- 2.2% control, 15 dps; -15.0% control, 30 dps).

Under pH-regime the depression-elevation pattern is found (\sim 4.6% control, 15 dps; + 18.0% control, 30 dps).

Under the combinational regime also the depression-elevation pattern is seen (\sim 15.0% control, 15 dps; + 23.6% control, 30 dps).

One important point that is highlighted by the histogrammetric data presented in VI.4 COMMENT VI.3 is the remarkable decrement in the weight status of hepatopancreas. This decrement is evident in both wet weight and dry weight and also in the water-somatic index (WSI).

The other three tissues studied, in contrast, exhibit the general trend of increase of the weight status under the influence of diverse stressants.

Of the three tissues, muscle shows moderate increments in wet and dry weight statuses whereas ovary shows remarkable increases of parameters.

This stressant-induced 'hypertrophy' observed in ovary makes a striking contrast to the 'hypotrophy' recorded in hepatopancreas and highlights the aspects of the basic biochemical 'personality' differences between the tissues.

These data on histogravimetry may be considered to add an additional quantitative dimension to the adversities caused by the stressants on crustaceans (Bernhard and Zattera, 1975; Vernberg et al., 1974; Lake and Thorp, 1974; Waldichuck, 1974).

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TABLE 6.1: Effect of individual and combined in vivo stress of Cd & pH on HSIs of wet weight of HP in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 8.012 \pm 0.932

Stress	15 d	Change % Control	30 d	Change % Control
Cd	3.650 \pm 1.07	- 54.4	5.800 \pm 2.04	- 27.6
pH	4.540 \pm 0.96	- 43.3	4.900 \pm 0.10	- 38.8
Combined (Comb)	5.007 \pm 1.72	- 37.5	4.210 \pm 1.83	- 47.5

TABLE 6.1a: Comparison of means of HSI's of wet weight of HP in O. senex senex with reference to stress conditions presented in Table 6.1.

F = 160.44

CD = 1.283

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	S	S	S	NS
pH 15	-	-	NS	NS	NS	NS
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	NS
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.2: Effect of individual and combined in vivo stress of Cd & pH on HSI's of dry weight of HP in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. 10 determinations).

Control : 3.250 ± 0.56

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.423 ± 0.81	- 56.3	2.700 ± 1.25	- 17.0
pH	1.900 ± 0.64	- 41.5	1.750 ± 0.42	- 46.0
Combined (Comb)	1.806 ± 0.90	- 44.4	1.410 ± 1.21	- 56.6

TABLE 6.2a: Comparison of means of HSIs of dry weight of HP in O. senex senex with reference to stress conditions presented in Table 6.2.

F = 69.35

CD = 0.782 .

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	S	NS	NS
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.3: Effect of individual and combined in vivo stress of Cd & pH on hydration level of HP in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 4.76 ± 0.76

Stress	15 d	Change % Control	30 d	Change % Control
Cd	2.23 ± 0.50	- 53.2	3.10 ± 0.87	- 34.9
pH	2.73 ± 0.27	- 42.7	3.16 ± 0.61	- 33.6
Combined (Comb)	3.20 ± 0.94	- 32.8	2.50 ± 0.20	- 47.5

TABLE 6.3a: Comparison of means of hydration level of HP in O. senex senex with reference to stress conditions presented in Table 6.3.

F = 281

CD = 0.581

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	S	S	S	NS
pH 15	-	-	NS	NS	NS	NS
Comb 15	-	-	-	NS	NS	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.I: Tissue weight-specific hydration levels (WSH) in the hepatopancreas (HP) of O. senex senex under individual and combined . in vivo regimes of Cd & pH.

Values, expressed as water (hydration) in g percent wet tissue weight are calculated from the data given in tables 6.1 and 6.3.

Control : 59.4				
Stress	15 d	Change % Control	30 d	Change % Control
Cd-treatment	61.1	+ 2.8	53.4	- 10.1
pH-treatment	60.1	+ 1.2	64.5	+ 8.6
Combined treatment	63.9	+ 7.6	59.4	0

TABLE 6.4: Effect of individual and combined in vivo stress of Cd & pH on HSI's of wet weight of M in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 17.02 \pm 1.00

Stress	15 d	Change % Control	30 d	Change % Control
Cd	18.97 \pm 1.03	+ 11.5	19.12 \pm 1.63	+ 12.3
pH	19.80 \pm 0.80	+ 16.3	18.80 \pm 0.70	+ 10.5
Combined (Comb)	18.60 \pm 0.64	+ 9.3	18.00 \pm 0.75	+ 5.8

TABLE 6.4a: Comparison of means of HSI's of wet weight of M in O. senex senex with reference to stress conditions presented in Table 6.4.

F = 4205

CD = 0.877

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	S	S	S
Cd 15	-	NS	NS	NS	NS	S
pH 15	-	-	S	NS	S	S
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.5: Effect of individual and combined in vivo stress of Cd & pH on HSIs of dry weight of M in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 8.760 ± 0.63

Stress	15 d	Change % Control	30 d	Change % Control
Cd	9.212 ± 0.26	+ 5.2	10.522 ± 1.86	+ 20.1
pH	9.300 ± 0.40	+ 6.2	10.570 ± 0.66	+ 20.0
Combined (Comb)	7.700 ± 1.54	- 12.0	8.000 ± 1.35	- 8.7

TABLE 6.5a: Comparison of means of HSIs of dry weight of M in O. senex senex with reference to stress conditions presented in Table 6.5.

F = 793

CD = 0.996

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	S	S	S	NS
Cd 15	-	NS	S	S	S	S
pH 15	-	-	S	S	S	S
Comb 15	-	-	-	S	S	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.6: Effect of individual and combined in vivo stress of Cd & pH on hydration level of M in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 8.250 ± 0.54

Stress	15 d	Change % Control	30 d	Change % Control
Cd	9.76 ± 1.04	+ 18.3	8.60 ± 1.86	+ 4.2
pH	10.50 ± 0.65	+ 27.3	8.30 ± 0.35	+ 0.6
Combined (Comb)	10.90 ± 1.60	+ 32.1	10.03 ± 1.45	+ 21.6

TABLE 6.6a: Comparison of means of hydration level of M in O. senex senex with reference to stress conditions presented in Table 6.6.

F = 765.73

CD = 1.152

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	S	S	NS	NS	S
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.II: Tissue weight-specific hydration levels (WSH) in the chelate leg muscle (M) of O. senex senex under individual and combined in vivo regimes of Cd and pH.
(Values, expressed as water (hydration) in g percent wet tissue weight, are calculated from the data given in tables 6.4 and 6.6.

Control : 48.5

Stress	15 d	Change % Control	30 d	Change % Control
Cd-treatment	51.4	+ 6.0	44.9	- 7.4
pH-treatment	53.0	+ 9.3	44.1	- 9.1
Combined treatment	58.6	+ 20.8	55.7	+ 14.8

TABLE 6.7: Effect of individual and combined in vivo stress of Cd & pH on HSI's of wet weight of G in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 1.430 ± 0.10

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.343 ± 0.50	- 6.0	2.500 ± 0.42	+ 74.8
pH	1.550 ± 0.20	+ 8.4	2.600 ± 0.16	+ 81.8
Combined (Comb)	1.560 ± 0.18	+ 9.1	1.415 ± 0.20	- 1.1

TABLE 6.7a: Comparison of means of HSIs of wet weight of G in O. senex senex with reference to stress conditions presented in Table 6.7.

F = 490

CD = 0.253

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	NS	S	S	NS
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.
CD value was calculated according to the formula
given in Table 3.1a.

TABLE 6.8: Effect of individual and combined in vivo stress of Cd & pH on HSIs of dry weight of G in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 0.180 ± 0.015

Stress	15 d	Change % Control	30 d	Change % Control
Cd	0.190 ± 0.067	+ 5.6	0.243 ± 0.040	+ 35.0
pH	0.200 ± 0.032	+ 11.1	0.173 ± 0.030	- 3.9
Combined (Comb)	0.231 ± 0.020	+ 28.3	0.203 ± 0.014	+ 12.8

TABLE 6.8a: Comparison of means of HSI's of dry weight of G in O. senex senex with reference to stress conditions presented in Table 6.8.

F = 387.1

CD = 0.0315

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	S	S	NS	NS
Cd 15	-	NS	S	S	NS	NS
pH 15	-	-	S	S	NS	NS
Comb 15	-	-	-	NS	S	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.9: Effect of individual and combined in vivo stress of Cd & pH on hydration level of G in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 1.250 \pm 0.09

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.154 \pm 0.43	- 7.7	2.243 \pm 0.40	+ 79.4
pH	1.360 \pm 0.13	+ 8.8	2.400 \pm 0.15	+ 92.0
Combined (Comb)	1.330 \pm 0.17	+ 6.4	1.211 \pm 0.20	- 3.1

TABLE 6.9a: Comparison of means of hydration level of G in O. senex senex with reference to stress conditions presented in Table 6.9.

F = 474.24

CD = 0.229

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	NS	NS	NS	S	S	NS
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S : Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.III: Tissue weight specific hydration levels (WSH) in the gill of O. senex senex under individual and combined in vivo regimes of Cd and pH.

Values, expressed as water (hydration) in g percent wet tissue weight, are calculated from the data given in tables 6.7 and 6.9.

Control : 87.4

Stress	15 d	Change % Control	30 d	Change % Control
Cd-treatment	85.9	- 1.7	89.7	+ 2.6
pH-treatment	87.7	+ 0.3	92.3	+ 5.6
Combined treatment	85.3	- 2.4	85.6	- 2.1

TABLE 6.10: Effect of individual and combined in vivo stress of Cd & pH on HSIs of wet weight of O in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 0.723 ± 0.40

Stress	15 d	Change % Control	30 d	Change % Control
Cd	3.34 ± 1.52	+ 362	1.76 ± 0.43	+ 143
pH	1.07 ± 0.35	+ 48.0	1.70 ± 0.31	+ 135
Combined (Comb)	1.20 ± 0.73	+ 66.0	0.55 ± 0.20	- 23.5

TABLE 6.10a: Comparison of means of HSI's of wet weight of O in O. senex senex with reference to stress conditions presented in Table 6.10.

F = 68.4

CD = 0.632

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	S	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	NS	NS	S
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.11: Effect of individual and combined in vivo stress of Cd & pH on HSIs of dry weight of O in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 0.300 ± 0.20

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.42 ± 0.60	+ 374	0.890 ± 0.23	+ 197
pH	0.500 ± 0.18	+ 66.7	0.534 ± 0.09	+ 78.0
Combined (Comb)	0.620 ± 0.34	+ 107	0.160 ± 0.08	- 46.7

TABLE 6.11a: Comparison of means of HSI's of dry weight of O in O. senex senex with reference to stress conditions presented in Table 6.11.

F = 73.6

CD = 0.263

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	S	S	NS	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	S
Comb 15	-	-	-	S	NS	S
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level, NS = Not Significant,

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.12: Effect of individual and combined in vivo stress of Cd & pH on hydration level of O in O. senex senex.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

Control : 0.425 ± 0.22

Stress	15 d	Change % Control	30 d	Change % Control
Cd	1.920 ± 0.94	+ 352	0.880 ± 0.21	+ 107
pH	0.600 ± 0.20	+ 41.2	1.150 ± 0.22	+ 171
Combined (Comb)	0.600 ± 0.40	+ 41.2	0.400 ± 0.18	- 5.9

TABLE 6.12a: Comparison of means of hydration level of O in O. senex senex with reference to stress conditions presented in Table 6.12.

F = 63.14

CD = 0.378

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
C	S	NS	NS	S	S	NS
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	NS	S	NS
Comb 15	-	-	-	NS	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.IV: Tissue weight-specific hydration levels (WSH) in the ovary (O) of O. senex senex under individual and combined in vivo regimes of Cd and pH.

Values expressed as water (hydration) in g percent wet tissue weight, are calculated from the data given in tables 6.10 and 6.12.

Control : 58.8

Stress	15 d	Change % Control	30 d	Change % Control
Cd-treatment	57.5	- 2.2	50.0	- 15.0
pH-treatment	56.1	- 4.6	67.6	+ 18.0
Combined treatment	50.0	- 15.6	72.7	+ 23.6

TABLE 6.13: Changes in the post-stress wet weight (PSWW) percent ante-stress wet weight (ASWW) of HP of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 8.01 ± 0.932

Treatment	Somatic weight (Experimental)	ASWW (Calculated)	PSWW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	3.321 ± 0.39	1.490 ± 0.35	- 54.4 ± 13.44
pH 15 d	27.7 ± 2.42	2.234 ± 0.20	1.286 ± 0.24	- 42.3 ± 9.00
Comb 15 d	24.3 ± 2.06	1.945 ± 0.16	1.220 ± 0.47	- 37.8 ± 21.50
Cd 30 d	32.9 ± 1.64	2.640 ± 0.13	1.898 ± 0.64	- 27.5 ± 31.30
pH 30 d	24.1 ± 1.98	1.950 ± 0.16	1.150 ± 0.15	- 40.1 ± 12.66
Comb 30 d	23.7 ± 1.87	1.820 ± 0.31	0.977 ± 0.36	- 48.0 ± 23.00

TABLE 6.13a: Comparison of means of changes in PSWW - ASWW of HP of O. senex senex with reference to stress conditions presented in Table 6.13.

F = 53.08

CD = 17.881

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	S	NS	NS
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.14: Changes in the post-stress dry weight (PSDW) percent ante-stress dry weight (ASDW) of HP of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 3.25 ± 0.560

Treatment	Somatic weight (Experimental)	ASWW (Calculated)	PSWW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	1.355 ± 0.16	0.567 ± 0.28	- 56.5 ± 24.68
pH 15 d	27.7 ± 2.42	0.905 ± 0.08	0.524 ± 0.16	- 41.6 ± 19.60
Comb 15 d	24.3 ± 2.06	0.800 ± 0.065	0.420 ± 0.262	- 47.0 ± 30.45
Cd 30 d	32.9 ± 1.64	1.073 ± 0.05	0.884 ± 0.40	- 17.7 ± 38.20
pH 30 d	24.1 ± 1.98	0.788 ± 0.063	0.412 ± 0.073	- 46.9 ± 12.80
Comb 30 d	23.7 ± 1.87	0.773 ± 0.061	0.320 ± 0.252	- 56.9 ± 37.00

TABLE 6.14a: Comparison of means of changes in PSDW % ASDW of HP of O. senex senex with reference to stress conditions presented in Table 6.14.

F = 31.46

CD = 25.653

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	S	NS	NS
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS : Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.15 Changes in the post-stress holohistontic hydration level (PSHHHL) percent ante-stress holohistontic, hydration level (ASHHHL) of HP of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 4.76 ± 0.760

Treatment	Somatic weight (Experimental)	ASHHHL (Calculated)	PSHHHL (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	1.966 ± 0.23	0.920 ± 0.22	- 53.0 ± 10.80
pH 15 d	27.7 ± 2.42	1.330 ± 0.12	0.763 ± 0.13	- 42.8 ± 5.43
Comb 15 d	24.3 ± 2.06	1.150 ± 0.10	0.800 ± 0.22	- 30.7 ± 16.81
Cd 30 d	32.9 ± 1.64	1.563 ± 0.08	1.014 ± 0.26	- 35.1 ± 17.66
pH 30 d	24.1 ± 1.98	1.162 ± 0.105	0.740 ± 0.104	- 35.4 ± 14.13
Comb 30 d	23.7 ± 1.87	1.146 ± 0.091	0.657 ± 0.136	- 42.0 ± 15.10

TABLE 6.15a: Comparison of means of changes in PSHHHL
% ASHHHL of HP of O. senex senex with
reference to stress conditions presented
in Table 6.15.

F = 101.27

CD = 12.495

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	S	S	S	NS
pH 15	-	-	NS	NS	NS	NS
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	NS
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 6.16: Changes in post-stress wet weight (PSWW) Percent ante-stress wet weight (ASWW) of M of O. senex senex with reference to individual and combined (Comb) in vivo treatment of Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 17.02 ± 1.00

Treatment	Somatic weight (Experimental)	ASWW (Calculated)	PSWW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	7.076 ± 0.79	7.806 ± 0.56	+ 10.9 ± 6.90
pH 15 d	27.7 ± 2.42	4.800 ± 0.43	5.473 ± 0.33	+ 14.6 ± 4.57
Comb 15 d	24.3 ± 2.06	4.133 ± 0.35	4.505 ± 0.35	+ 9.2 ± 3.71
Cd 30 d	32.9 ± 1.64	5.607 ± 0.28	6.276 ± 0.55	+ 12.3 ± 9.60
pH 30 d	24.1 ± 1.98	4.099 ± 0.34	4.515 ± 0.30	+ 10.4 ± 4.30
Comb 30 d	23.7 ± 1.87	4.065 ± 0.31	4.260 ± 0.45	+ 4.6 ± 4.71

TABLE 6.16a: Comparison of means of changes in PSWW % ASWW of M of Q. senex senex with reference to stress conditions presented in Table 6.16.

F = 41.2

CD = 5.207

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	NS	NS	S
pH 15	-	-	S	NS	NS	S
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.17: Changes in post-stress dry weight (PSDW) percent ante-stress dry weight (ASDW) of M of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 8.76 ± 0.630

Treatment	Somatic weight (Experimental)	ASDW (Calculated)	PSDW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	3.636 ± 0.43	3.812 ± 0.46	+ 4.8 ± 2.52
pH 15 d	27.7 ± 2.42	2.435 ± 0.21	2.565 ± 0.20	+ 5.6 ± 4.50
Comb 15 d	24.3 ± 2.06	2.140 ± 0.18	1.890 ± 0.50	- 12.7 ± 17.50
Cd 30 d	32.9 ± 1.64	2.913 ± 0.16	3.175 ± 1.07	+ 18.6 ± 20.16
pH 30 d	24.1 ± 1.98	2.124 ± 0.16	2.530 ± 0.23	+ 19.1 ± 7.85
Comb 30 d	23.7 ± 1.87	2.090 ± 0.16	1.870 ± 0.25	- 9.9 ± 15.13

TABLE 6.17a: Comparison of means of changes in PSDW % ASDW of M of O. senex senex with reference to stress conditions presented in Table 6.17.

F = 12.00

CD = 11.747

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	NS
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.18: Changes in post-stress holohistontic hydration level (PSHHHL) percent ante-stress holohistontic hydration level (ASHHHL) of M of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 8.25 ± 0.540

Treatment	Somatic weight (Experimental)	ASHHHL (Calculated)	PSHHHL (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	3.445 ± 0.36	4.009 ± 0.21	+ 17.3 ± 11.41
pH 15 d	27.7 ± 2.42	2.365 ± 0.24	2.907 ± 0.18	+ 23.7 ± 8.70
Comb 15 d	24.3 ± 2.06	2.000 ± 0.17	2.615 ± 0.16	+ 32.5 ± 19.42
Cd 30 d	32.9 ± 1.64	2.700 ± 0.14	2.801 ± 0.50	+ 5.0 ± 22.13
pH 30 d	24.1 ± 1.98	1.980 ± 0.20	1.989 ± 0.10	+ 1.1 ± 6.70
Comb 30 d	23.7 ± 1.87	1.974 ± 0.15	2.386 ± 0.50	+ 20.3 ± 18.40

TABLE 6.18a: Comparison of means of changes in PSHHHL
% ASHHHL of M of O. senex senex with
reference to stress conditions presented
in Table 6.18.

F = 19.46

CD = 13.964

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	S	NS	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 6.19: Changes in post-stress wet weight (PSWW) percent ante-stress wet weight (ASWW) of G of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 1.43 ± 0.100

Treatment	Somatic weight (Experimental)	ASWW (Calculated)	ASWW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	0.590 ± 0.070	0.542 ± 0.166	- 5.8 ± 34.54
pH 15 d	27.7 ± 2.42	0.395 ± 0.035	0.430 ± 0.045	+ 8.1 ± 8.10
Comb 15 d	24.3 ± 2.06	0.350 ± 0.029	0.374 ± 0.021	+ 9.0 ± 12.60
Cd 30 d	32.9 ± 1.64	0.470 ± 0.023	0.813 ± 0.143	+ 73.2 ± 28.90
pH 30 d	24.1 ± 1.98	0.343 ± 0.028	0.616 ± 0.036	+ 80.2 ± 11.26
Comb 30 d	23.7 ± 1.87	0.340 ± 0.027	0.335 ± 0.066	- 2.2 ± 13.13

TABLE 6.19a: Comparison of means of changes in PSWW % ASWW of G of O. senex senex with reference to stress conditions presented in Table 6.19

F = 55.0

CD = 19.201

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.20: Changes in post-stress dry weight (PSDW) percent ante-stress dry weight (ASDW) of G of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams are mean \pm S.D. of 10 determinations).

HSI of normal = 0.180 ± 0.015

Treatment	Somatic weight (Experimental)	ASDW (Calculated)	PSDW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	0.074 ± 0.008	0.076 ± 0.022	+ 5.3 ± 37.10
pH 15 d	27.7 ± 2.42	0.050 ± 0.004	0.053 ± 0.010	+ 6.2 ± 17.20
Comb 15 d	24.3 ± 2.06	0.043 ± 0.003	0.056 ± 0.005	+ 28.7 ± 10.43
Cd 30 d	32.9 ± 1.64	0.059 ± 0.002	0.080 ± 0.014	+ 34.7 ± 22.25
pH 30 d	24.1 ± 1.98	0.043 ± 0.003	0.041 ± 0.007	- 4.0 ± 15.40
Comb 30 d	23.7 ± 1.87	0.043 ± 0.003	0.050 ± 0.007	+ 13.7 ± 8.32

TABLE 6.20a: Comparison of means of changes in PSDW %
 ASDW of G of 0. senex senex with reference
 to stress conditions presented in Table 6.20.

F = 10.7

CD = 18.593

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	S	S	NS	NS
pH 15	-	-	S	S	NS	NS
Comb 15	-	-	-	NS	S	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
 given in Table 3.1a.

TABLE 6.21: Changes in post-stress holohistontic hydration level (PSHHHL) percent ante-stress holohistontic hydration level (ASHHHL) of G of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations)

HSI of normal = 1.250 ± 0.087

Treatment	Somatic weight (Experimental)	ASHHHL (Calculated)	PSHHHL (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	0.516 ± 0.060	0.465 ± 0.143	- 7.4 ± 34.32
pH 15 d	27.7 ± 2.42	0.345 ± 0.030	0.374 ± 0.035	+ 8.2 ± 11.15
Comb 15 d	24.3 ± 2.06	0.303 ± 0.026	0.318 ± 0.022	+ 6.2 ± 13.9
Cd 30 d	32.9 ± 1.64	0.410 ± 0.020	0.734 ± 0.136	+ 79.0 ± 31.70
pH 30 d	24.1 ± 1.98	0.300 ± 0.025	0.570 ± 0.030	+ 92.7 ± 11.70
Comb 30 d	23.7 ± 1.87	0.296 ± 0.023	0.287 ± 0.060	- 3.8 ± 14.32

TABLE 6.21a: Comparison of means of changes in PSHHHL
% ASHHHL of G of O. senex senex with refe-
rence to stress conditions presented in
Table 6.21.

F = 64.41

CD = 19.363

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	NS	NS	S	S	NS
pH 15	-	-	NS	S	S	NS
Comb 15	-	-	-	S	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 6.22: Changes in post-stress wet weight (PSWW) percent ante-stress wet weight (ASWW) of 0 of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 0.723 ± 0.400

Treatment	Somatic weight (Experimental)	ASWW (Calculated)	PSWW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	0.302 ± 0.035	1.359 ± 0.620	+ 356 ± 206
pH 15 d	27.7 ± 2.42	0.200 ± 0.017	0.288 ± 0.077	+ 47.2 ± 48.40
Comb 15 d	24.3 ± 2.06	0.176 ± 0.015	0.277 ± 0.150	+ 64.7 ± 100
Cd 30 d	32.9 ± 1.64	0.240 ± 0.014	0.582 ± 0.160	+ 145 ± 60.6
pH 30 d	24.1 ± 1.98	0.174 ± 0.014	0.400 ± 0.040	+ 132 ± 42.63
Comb 30 d	23.7 ± 1.87	0.172 ± 0.014	0.130 ± 0.051	- 25.5 ± 26.55

TABLE 6.22a: Comparison of means of changes in PSWW %
ASWW of 0 of O. senex senex with reference
to stress conditions presented in Table 6.22

F = 33.85

CD = 90.479

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	NS	NS	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

TABLE 6.23: Changes in post-stress dry weight (PSDW) percent ante-stress dry weight (ASDW) of 0 of O. senex senex with reference to individual and combined

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 0.300 ± 0.190

Treatment	Somatic weight (Experimental)	ASDW (Calculated)	PSDW (Actual)	Change post-stress % ante-stress
Cd 15 d	41.4 ± 4.84	0.125 ± 0.015	0.580 ± 0.246	+ 370 ± 197
pH 15 d	27.7 ± 2.42	0.084 ± 0.007	0.133 ± 0.038	+ 63.26 ± 58.40
Comb 15 d	24.3 ± 2.06	0.073 ± 0.006	0.141 ± 0.070	+ 100 ± 112
Cd 30 d	32.9 ± 1.64	0.099 ± 0.005	0.300 ± 0.085	+ 193 ± 80.0
pH 30 d	24.1 ± 1.98	0.072 ± 0.006	0.125 ± 0.012	+ 75.72 ± 32.7
Comb 30 d	23.7 ± 1.87	0.073 ± 0.005	0.036 ± 0.017	- 50.66 ± 24.73

TABLE 6.23a: Comparison of means of changes in PSDW
% ASDW of 0 of O. senex senex with reference to stress conditions presented in Table 6.23.

F = 37.3

CD = 92.023

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	S	NS	NS
Comb 15	-	-	-	S	NS	NS
Cd 30	-	-	-	-	S	S
pH 30	-	-	-	-	-	NS

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula given in Table 3.1a.

TABLE 6.24: Changes in post-stress holohistontic hydration level (PSHHHL) percent ante-stress holohistontic hydration level (ASHHHL) of 0 of O. senex senex with reference to individual and combined (Comb) in vivo treatment with Cd & pH.

(Values, expressed as grams, are mean \pm S.D. of 10 determinations).

HSI of normal = 0.425 ± 0.219

Treatment	Somatic weight (Experimental)	ASHHHL (Calculated)	PSHHHL (Actual)	Change post-stress % ante-s
Cd 15 d	41.4 ± 4.84	0.177 ± 0.020	0.780 ± 0.380	+ 290 ± 205
pH 15 d	27.7 ± 2.42	0.116 ± 0.011	0.154 ± 0.042	+ 35.67 ± 44.00
Comb 15 d	24.3 ± 2.06	0.103 ± 0.010	0.136 ± 0.082	+ 39.20 ± 93.67
Cd 30 d	32.9 ± 1.64	0.138 ± 0.010	0.293 ± 0.078	+ 112 ± 50.65
pH 30 d	24.1 ± 1.98	0.102 ± 0.007	0.273 ± 0.027	+ 171 ± 49.50
Comb 30 d	23.7 ± 1.87	0.104 ± 0.016	0.095 ± 0.046	- 7.31 ± 41.75

TABLE 6.24a: Comparison of means of changes in PSHHHL
% ASHHHL of O of O. senex senex with refe-
rence to stress conditions presented in
Table 6.24.

F = 19.42

CD = 98.531

Comparison of	With					
	Cd 15	pH 15	Comb 15	Cd 30	pH 30	Comb 30
Cd 15	-	S	S	S	S	S
pH 15	-	-	NS	NS	S	NS
Comb 15	-	-	-	NS	S	NS
Cd 30	-	-	-	-	NS	S
pH 30	-	-	-	-	-	S

S = Significant at 5% level; NS = Not Significant.

CD value was calculated according to the formula
given in Table 3.1a.

Fig. 6.1: Percent change in wet weight of hepatopancreas (HP) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

Fig. 6.2: Percent change in dry weight of hepatopancreas (HP) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and C their combinational (Comb.) concentration states.

Fig. 6.3: Percent change in hydration level (HHHL) of hepatopancreas (HP) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

Fig. 6.4: Percent change in wet weight of muscle (M) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

FIG:6-1

HP WET WT.

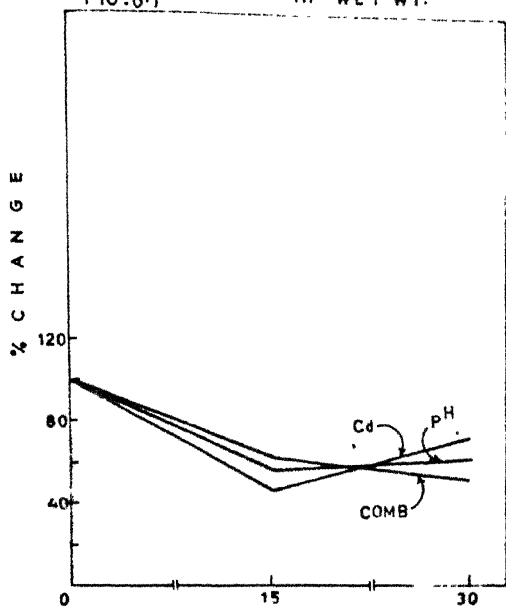


FIG 6-2

HP DRY WT.

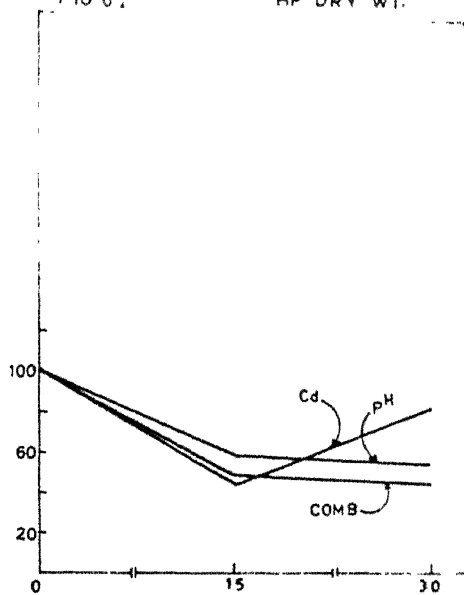


FIG:6-3

HP HHHL

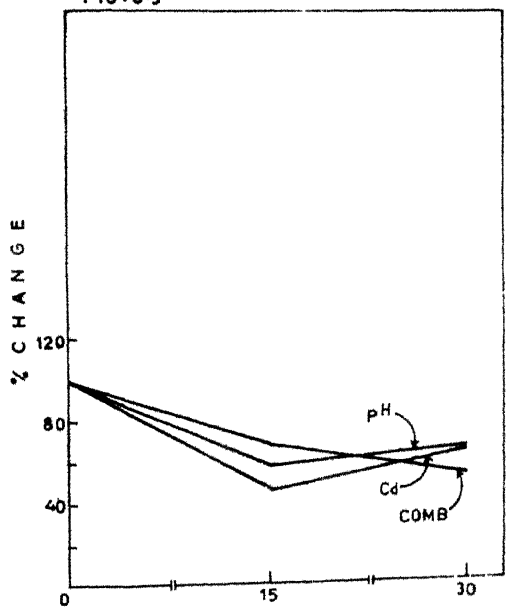


FIG:6-4

M WET WT.

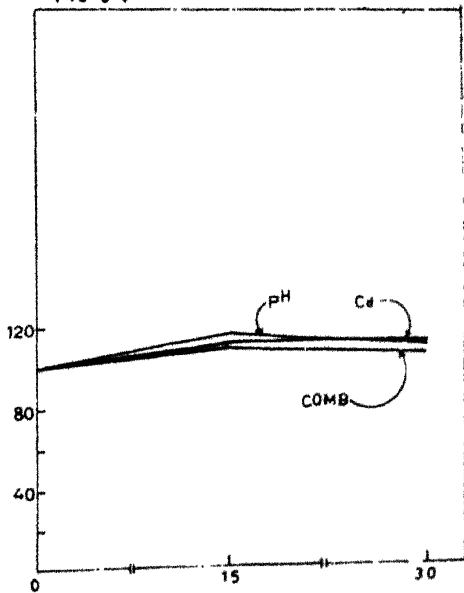
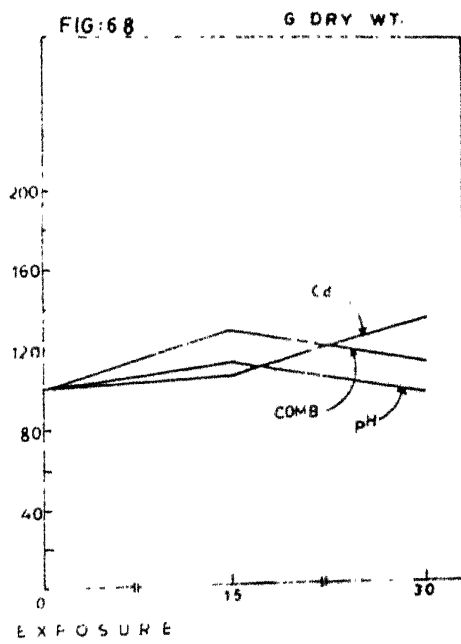
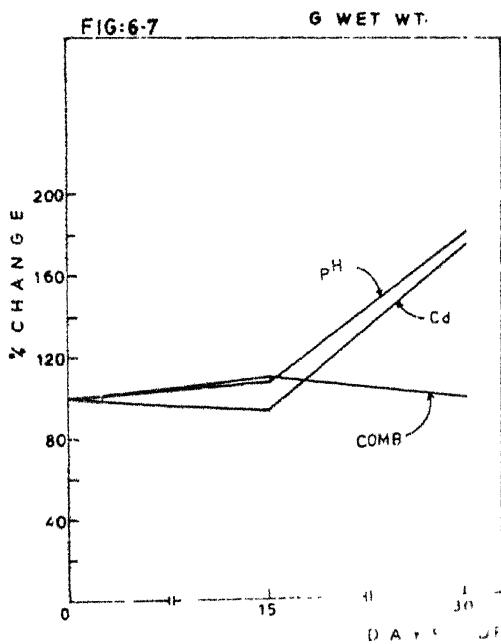
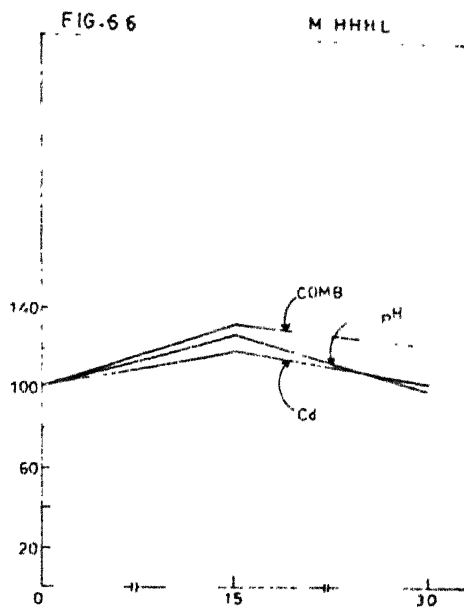
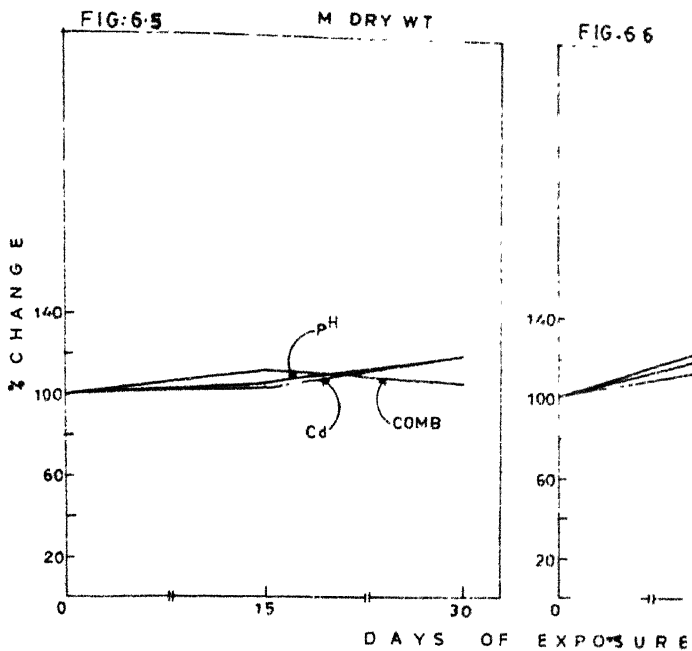


Fig. 6.5: Percent change in dry weight of muscle (M) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

Fig. 6.6: Percent change in hydration level (HIL) of muscle (M) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

Fig. 6.7: Percent change in wet weight of gill (G) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

Fig. 6.8: Percent change in dry weight of gill (G) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.



- Fig. 6.9 : Percent change in hydration level (HHHL) of gill (G) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.
- Fig. 6.10: Percent change in wet weight of ovary (O) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.
- Fig. 6.11: Percent change in dry weight of ovary (O) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.
- Fig. 6.12: Percent change in hydration level (HHHL) of ovary (O) of crabs at 15 d and 30 d sublethal treatments with Cd-, pH- and their combinational (Comb.) concentration states.

FIG:6-9

G HHHL

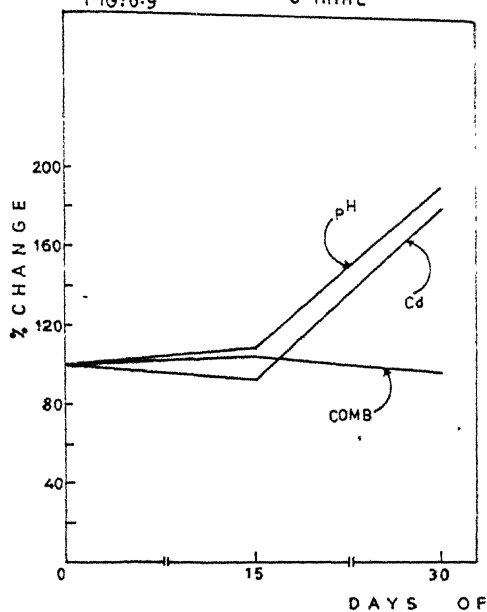


FIG:6-10

O WET WT.

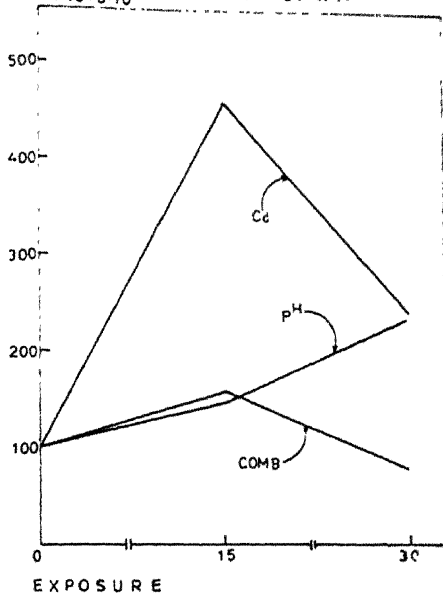


FIG:6-11

O DRY WT.

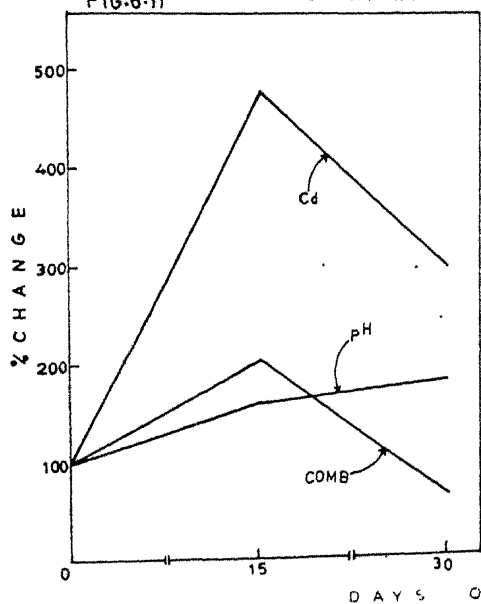


FIG:6-12

O HHHL

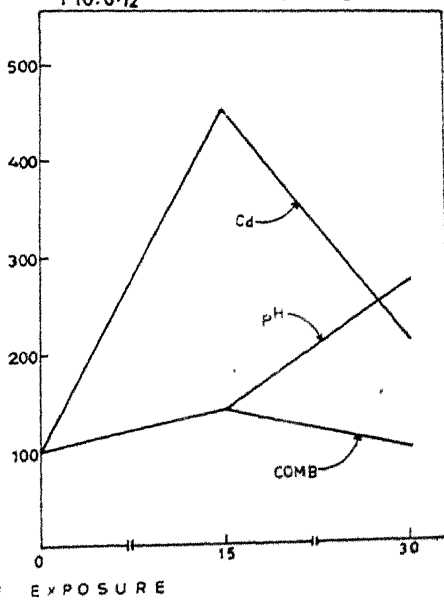
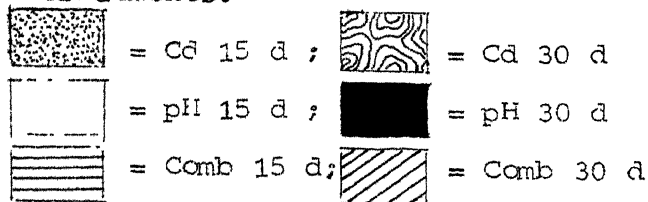


Fig. 6.13: Histograms showing percent change in wet weight of hepatopancreas (HP) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.14: Histograms showing percent change in dry weight of hepatopancreas (HP) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.15: Histograms showing percent change in hydration level (HHHL) of hepatopancreas (HP) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.16: Histograms showing percent change in wet weight of muscle (M) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.



NL = Normal line; above NL (+) = increment and below NL (-) = decrement.

FIG:6-13

HP WET WT-

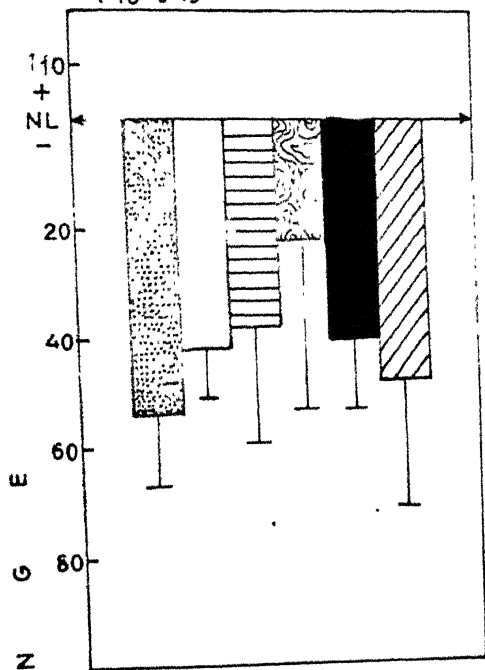


FIG:6-14

HP DRY WT

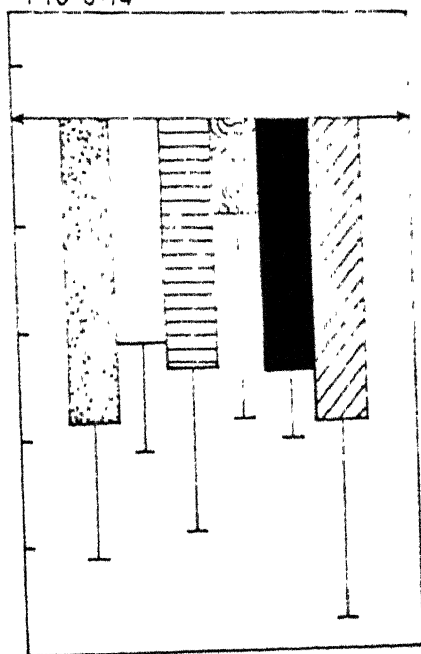


FIG:6-15

HP HHHL

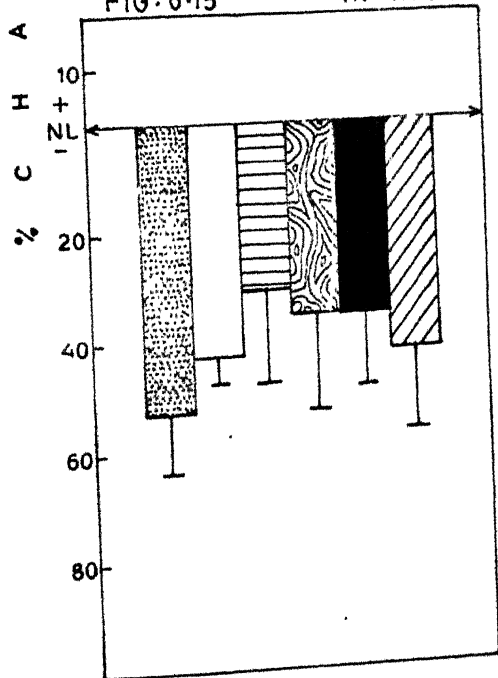


FIG:6-16

M WET WT

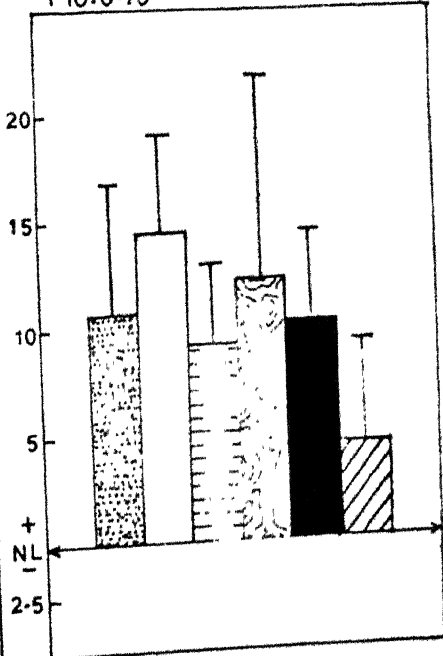
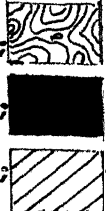



Fig. 6.17: Histograms showing percent change in dry weight of muscle (M) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.


Fig. 6.18: Histograms showing percent change in hydration level (HHHL) of muscle (M) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.


Fig. 6.19: Histograms showing percent change in wet weight of gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments

Fig. 6.20: Histograms showing percent change in dry weight of gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.



 = Cd 15 d ;  = Cd 30 d

 = pH 15 d ;  = pH 30 d

 = Comb 15 d ;  = Comb 30 d

NL = Normal line; above NL(+) = increment and below NL(-) = decrement.

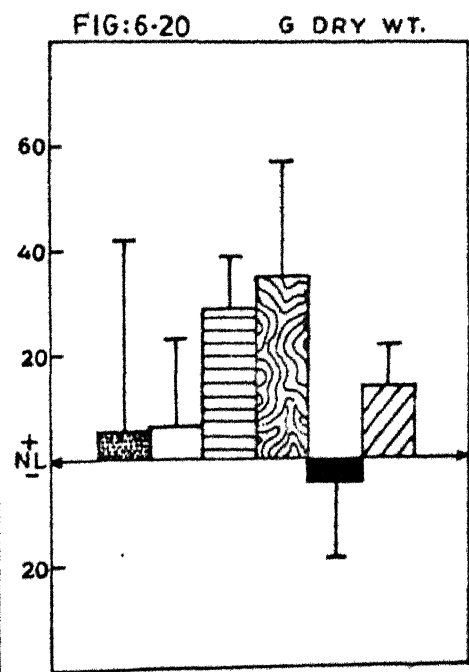
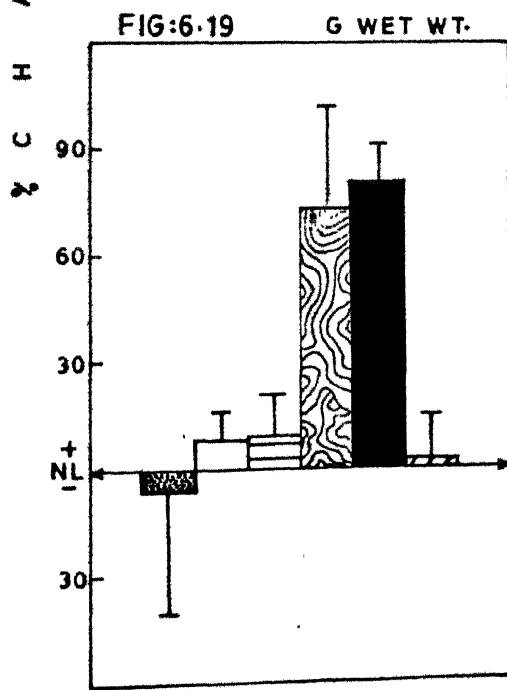
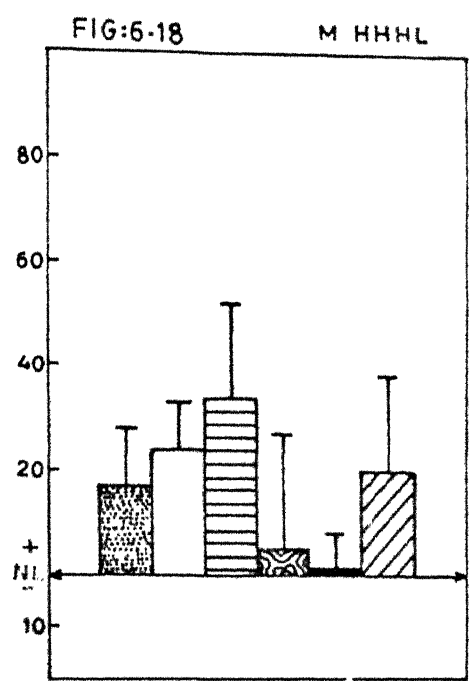
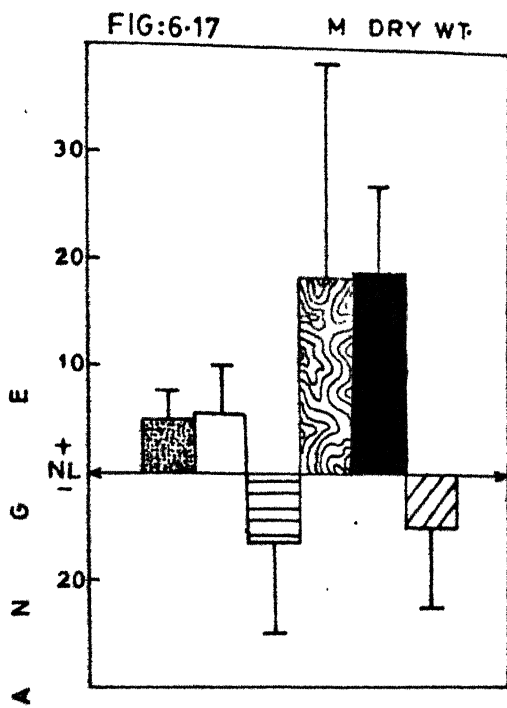
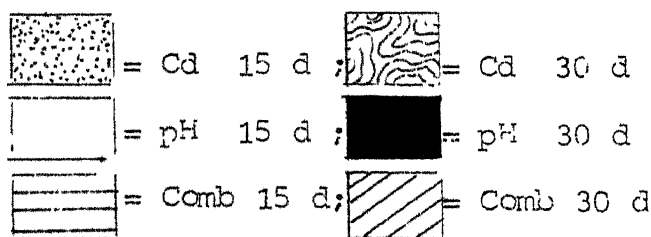


Fig. 6.21: Histograms showing percent change in hydration level (HHHL) of gill (G) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.22: Histograms showing percent change in wet weight of ovary (O) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.23: Histograms showing percent change in dry weight of ovary (O) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.

Fig. 6.24: Histograms showing percent change in hydration level (HHHL) of ovary (O) of Cd-, pH- and combinationally (Comb.) intoxicated crabs at 15 d and 30 d sublethal treatments.



NL = Normal line; above NL (+) = increment and below NL(-) = decrement.

FIG:6-21

G HHHL

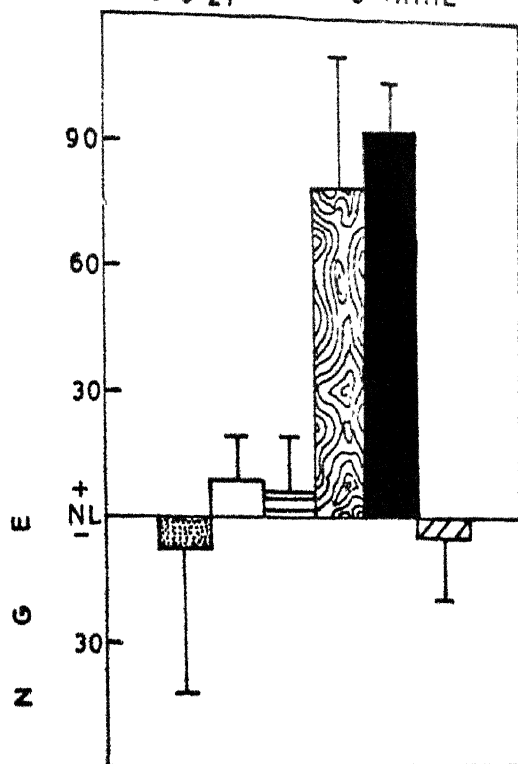


FIG:6-22

O WET WT.

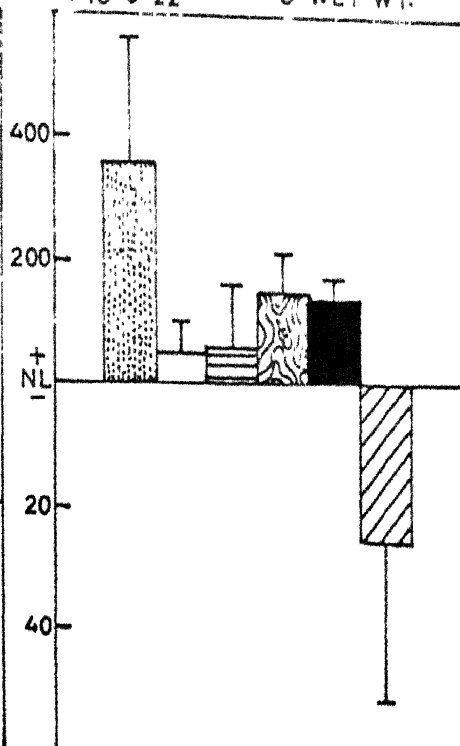


FIG:6-23

O DRY WT.

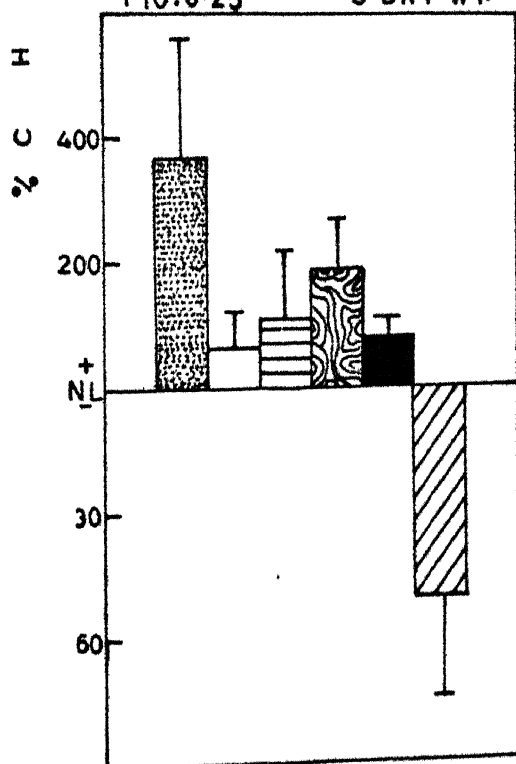
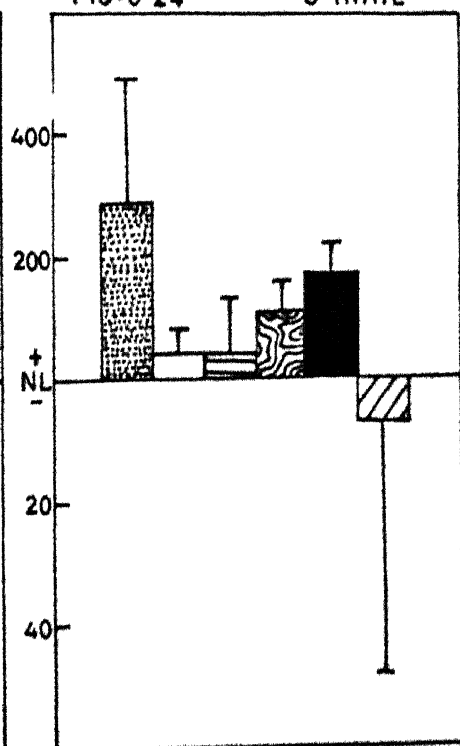


FIG:6-24

O HHHL



CHAPTER VI

CALCULATIONAL GRAVIMETRY

In the preceding chapteral location (Chapter VI) the aspect of histogravimetry has been examined in some detail. This examination has proven useful in revealing certain interesting trends of change caused by the stressants in the 'tissue biology' of the organism.

In the present chapteral location, a 'calculational exercise' will be undertaken to examine the usefulness of a new elucidation methodology which is named 'calculative histometry'.

This method in essence involves calculation of antestress weights of tissues in individuals, subjected to the different stressant treatments.

The procedural steps regarding the preparation of the control and experimental groups of crabs are given elsewhere.

VI 2 MATERIAL AND METHOD

But, with regard to calculative histometry the method of stressing the organism will be a bit different. In the histogravimetry work, usually a batch (experimental/control) is kept in an aquarium without any individual identification or locational demarkation. But in the

calculative histometric approach each individual is kept in a separate stressant container. Alternatively each individual crab is marked for identification using water-proof paint. The weights of the marked individuals are recorded, prior to the moment of placing them in the stressant-containers. These weights are designated ante-stress somatic weights (ASSW).

The individuals are allowed the given durations of stress and at the end of these durations (Viz., shorter stress-duration, 15 dps; longer stress-duration, 30 dps) are sacrificed after their somatic weights are noted (the post-stress somatic weight, PSSW). Incidentally this approach of recording ASSWs and PSSWs immediately gives information about the influence of the stressant in question on the somatic weight status of the individuals.

In the sacrificed organisms, post-stress, the tissues are isolated (as detailed in Chapter VI) and their weights are recorded (post-stress tissue weights, PSTW).

In another experiment, a batch of normal, unstressed crabs is used for the determination of normal (= control) histosomatic indices of the tissues.

The 'normal' HSI of a given tissue, divided into 100, gives a factor, F.

$$F = \text{HSI}/100 \quad \dots \quad (1)$$

This factor, when multiplied with the ante-stress somatic weight gives the calculated ante-stress tissue weight (CASTW).

$$\text{ASSW} \times F = \text{CASTW} \quad \dots \quad (2)$$

The difference between CASTW and PSTW gives more meaningful picture of change of the tissue-weight status under the stressant-regimes.

$$\text{CASTW} - \text{PSTW} = \text{TW}_{\text{calc}} \quad \dots \quad (3)$$

(where TW_{calc} stands for the change in tissue weight obtained by calculative histometric approach).

The calculative histometric approach can be extended to the tissue dry weight parameter and tissue hydration parameter. So much so, these parameters too will obtain additional elucidative perspective.

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595.3842
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VI. 3 RESULTS

The changes in somatic weights followed in individual organisms are small and statistically non-significant (Table 6.V).

It should be clear from these data that the somatic weight status of the organism is unaffected by the various stressant regimes.

The results of the calculational gravimetric exercise carried on the tissues of O. senex senex subjected to diverse stressant media are tabulated in tables 5.13 to 6.24. The statistical treatment of these data are given in tables 6.13a....6.24a et seq.

Tables 6.13 to 6.15 give the data for hepato-pancreatic tissue; tables 6.16 to 6.18 for the chelate leg muscle; tables 6.19 to 6.21 for gill; and 6.22 to 6.24 for ovary.

The parameters that have been examined earlier viz., tissue wet weight, dry weight and hydration levels are once again been examined here, under calculational gravimetric focus.

One point that emerges from this examination is that calculational histometric approach yields essentially the same insights into the influence of the

stressants on the tissue weight status, as have been obtained according to the plain histogravimetric approach involving HSI determinations and normal and stressant-treated organisms.

The present sub-chapter
 VI & 4 COMMENT is intended primarily to
 introduce the concept and practice of
 calculative gravimetry in the field of toxico-physiological and biochemical enquiry.

That this exercise has not improved the insights obtained by histogravimetric enquiry may not detract from the value of this approach in this field of investigation. Especially, this calculative gravimetric approach allows one to examine, with a fair extent of reliability, the gravimetric drama of a tissue in a given individual under a given stress. Provided the index of the tissue in question is determined from a reasonably large and appropriate sample, one can use this approach to visualize tissue gravimetric drama without the spectacle of statistics.

TABLE 6.V: Changes in somatic weight, post-stress (PSSW) as compared to the antestress somatic weight, of the crabs exposed to different stressant media.

Stress	ASSW	PSSW	Change PSSW % ASSW	t	p
Cd 15 d	41.4 \pm 4.84 (10)	42.1 \pm 5.18 (10)	+ 1.7	0.07	NS
pH 15 d	27.7 \pm 2.42 (10)	26.8 \pm 3.18 (10)	- 3.2	0.30	NS
Comb 15 d	24.3 \pm 2.06 (10)	24.8 \pm 2.19 (10)	+ 2.1	0.06	NS
Cd 30 d	32.9 \pm 1.64 (10)	32.6 \pm 1.82 (10)	- 0.9	0.10	NS
pH 30 d	24.1 \pm 1.98 (10)	25.3 \pm 1.80 (10)	+ 5.0	0.09	NS
Comb 30 d	23.7 \pm 1.87 (10)	24.2 \pm 1.92 (10)	+ 2.1	0.03	NS

Values, expressed in grams, are mean \pm S.D. of 10 experiments.

t = calculated students 't' test value; P = level of significance.

NS = Not Significant.

CHAPTER VII

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DISCUSSION

CHAPTERULE VII 1

PREFATORY

in the earlier chapteral locations
in this dissertation, the stressants
pH and Cd have been visualised to
cause alterations in the metabolic
rate of the crab Oziotelphusa senex
senex in severo and in combinatio.
The scenario of the organismic meta-
bolism under the stressant regimes
has been found to be characterised

by depression of oxygen consumption (Chapter III) which accords well with literature reports indicating depression of organismic metabolism as the general stress-induced 'pathophany' (pathological show-up).

The organic (Chapter IV) and macromolecular catalytic (Chapter V) composition of the tissues of the crab under the stressant regimes appears to follow this theme of 'tissue hypoxia'.

What is the metabolic profile of this hypoxia ?

The stressants cause changes in tissue metabolism, in different quantal dimensions in their individual and combinational regimes. Do the stressants in the combinational regimes exert influence over one another? In other words, do they 'interact' with one another in the modes of pathogeny?

These and more questions may have agitated the mind of the reader as he has perused the data presented in the earlier chapteral locations.

In this terminal chapteral location, efforts will be directed to build up a theme of pathophany which will satiate some if not all of the quests of the inquirer.

CHAPTER VII 2

DATAL RETROSPECT

In the crab O. senex senex the stressants Cd and pH cause alterations in the levels of tissue biochemical components and macromolecular catalytic potentialities. They also lead to alterations in the weight and hydration profiles of the tissues. The data given in earlier chapteral loca-

tions on these aspects of 'tissue biology' vis-a-vis the stressants are consolidated here, as a prelude to an in depth analysis of the implications of these 'datal details' (Table VII 2.1).

From this vantage point, one may embark on the examination of several 'extractions' that result from a closer scrutiny of the datal retrospect.

One such extraction pertains to certain ratio-relations between the biochemical and catalytic components of the tissues.

			C3						C4						C5						C6		
			30 d			15 d			30 d			15 d			30 d			15 d			30 d		
			% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	% C	
6	TP	21	420	100.0	330	150.0	261	100.0	382	172.6	275	124.2	315	142.5									
	SP	180	210	121.4	216	120.0	214	118.4	250	138.7	216	120.0	240	133.4									
	ISI	41	30	493	114	278	47.0	114.6	132	322	99.0	183.4	75.0	102.9									
	TPCHR	70.1	13.7	86.2	28.7	72.4	76.2	67.0	27.1	69.3	25.2	56.7	33.0	67.7									
	TAPAPS	10.0	15.6	156.0	18.3	183.0	12.6	43.4	16.0	55.0	14.4	50.0	32.0	116.0									
	TAPPS	235	287	122.0	251	109.0	13.6	113.6	11.1	111.0	7.8	78.0	6.0	60.0									
	TL	112	156	138.0	120	107.2	180	160.0	143	130.0	210	89.2	377	160.2									
	T-ATPase	1.620	3.140	194.0	1.040	64.2	1.700	105.0	0.800	50.0	1.780	109.6	4.010	248									
	Mg ²⁺ -ATPase	0.717	1.530	213	0.563	78.5	1.200	167.4	0.453	63.2	1.042	145.3	1.300	181.3									
	Non-Mg ²⁺ -ATPase	0.903	1.610	177.0	0.477	53.0	0.500	55.0	0.347	38.2	1.042	145.3	1.300	181.3									
	AAT	0.763	4.000	464	1.030	119.4	0.913	105.8	3.000	348	1.430	165.7	3.000	348									
	ALAT	0.565	2.500	443	0.660	117.0	0.506	88.5	2.800	496	1.000	177.0	2.000	390									
	SDH	0.275	1.530	556	0.666	242	0.338	120.0	0.427	155.3	0.266	96.7	0.300	109.1									
	LDH	0.121	0.578	478	0.240	198.3	0.130	107.4	0.044	76.4	0.130	107.4	0.200	165.3									
	C10H	MDH	0.134	0.524	391	0.207	154.5	0.107	80.0	0.100	74.6	0.118	88.0	0.233	174.0								
Wet HSI		4.012	3.650	46.6	5.800	72.4	4.540	56.7	4.900	61.2	5.007	62.5	4.210	52.5									
Dry HSI		3.250	1.423	43.8	2.700	83.1	1.900	58.5	1.750	53.8	1.906	55.6	1.410	43.4									
WSI		4.76	2.73	46.8	3.100	65.1	2.73	57.4	3.160	66.4	3.200	67.2	2.500	52.5									
TP		191	250	131.0	210	110.0	188	96.6	280	146.4	246	128.5	328	171.3									
SP		153	171	111.7	123	80.2	151	93.4	215	140.5	198	129.0	250	98.4									
ISI		38	79	208	87	229	37	97.4	65	171.1	48	126.3	178	466									
TPCHR		40.9	24.7	60.4	30.5	74.6	35.0	85.6	23.5	57.5	20.5	50.1	16.5	40.3									
TAPAPS		25.4	9.0	31.4	17.5	68.6	14.3	56.0	15.0	59.0	10.0	40.0	8.2	32.0									
TAPPS		15.4	16.7	109.0	13.0	84.4	20.7	134.4	8.5	55.2	10.5	68.2	6.3	54.0									
TNPS		300	363	121.1	329	110.0	480	160.6	30.7	102.3	315	105.1	377	126.0									
TL		66.8	49.2	73.8	60.3	90.4	17.5	26.3	36.6	54.9	96.5	144.5	68.6	102.7									
T-ATPase		0.922	0.550	60.0	0.650	70.5	1.124	122.0	0.400	43.4	1.000	108.5	1.650	179.0									
Mg ²⁺ -ATPase		0.600	0.423	70.5	0.298	50.0	0.730	78.0	0.325	54.8	0.611	107.8	0.725	121.0									
Non-Mg ²⁺ -ATPase		0.322	0.127	39.2	0.352	109.4	0.394	102.0	0.075	27.6	0.389	43.0	0.925	15.0									
C10H	SDH	0.983	0.513	52.8	0.341	34.3	1.002	102.0	0.065	67.6	0.424	43.0	0.150	11.0									
	LDH	0.274	0.134	49.0	0.107	39.0	0.088	31.4	0.103	37.6	0.120	40.0	0.030	11.0									
	GDH	0.625	0.302	48.3	0.250	40.0	0.302	48.3	0.610	97.6	0.362	58.0	0.023	3.7									
	MDH	0.353	0.307	87.0	0.277	78.0	0.340	96.3	0.400	113.3	0.090	25.5	0.090	25.5									
	Wet HSI	1.430	1.343	93.9	2.500	174.8	1.550	106.4	2.600	181.8	1.560	109.1	1.415	99.0									
	Dry HSI	0.180	0.190	105.6	0.243	135.0	0.200	111.1	0.173	96.1	0.231	128.3	0.203	112.8									
	WSI	1.250	1.154	92.3	2.243	179.4	1.360	108.8	2.400	192.0	1.330	106.4	1.211	96.9									
	TP	150	400	266	202	134.8	300	200	260	173.3	351	234	280	186.6									
	SP	120	107	89.2	110	91.6	162	135.0	230	192.0	136	113.4	250	208									
	ISI	30	293	977	92	307	138	460	30	100	115	383	30	100									
	TPCHR	32.0	29.3	91.6	40.1	125.3	27.4	85.6	22.5	70.3	30.5	95.3	18.3	57.2									
	TAPAPS	21.0	12.0	57.3	24.6	117.2	13.0	62.0	16.5	78.6	10.5	50.0	12.3	58.6									
	TAPPS	11.0	17.3	157.6	15.5	141.0	14.4	131.0	6.0	55.5	20.0	182.0	6.0	55.5									
	TNPS	451	732	162.2	306	67.7	527	117.0	433	96.0	358	80.0	381	84.5									
	TL	68.8	30.8	44.8	50.9	73.9	33.3	48.5	53.2	77.3	34.8	50.6	54.6	79.4									
ACHS	3.25	3.60	110.8	3.81	117.2	2.34	72.0	4.30	132.3	3.40	104.6	9.00	277										
T-ATPase	0.254	0.265	112.2	0.183	72.0	0.350	137.8	0.185	72.8	0.263	103.5	1.301	512										
Mg ²⁺ -ATPase	0.133	0.264	198.5	0.122	91.7	0.330	248	0.126	94.7	0.260	195.5	0.614	462										
Non-Mg ²⁺ -ATPase	0.121	0.021	17.4	0.061	50.4	0.020	16.5	0.059	48.8	0.003	2.5	0.687	568										
HL	AAT	0.300	0.305	102.0	0.311	104.0	0.031	10.3	1.200	400	0.520	173.3	1.204	401									
	ALAT	0.200	0.173	86.5	0.140	70.0	0.063	31.5	1.006	503	0.167	80.5	0.900	450									
	SDH	0.471	0.180	38.2	0.220	46.7	0.241	51.2	0.120	25.5	0.360	80.6	0.220	46.7									
	LDH	0.262	0.121	46.2	0.162	39.0	0.054	20.6	0.150	57.2	0.074	28.2	0.500	191.0									
	GDH	0.260	0.065	25.0	0.085	32.7	0.114	44.0	0.142	54.6	0.240	82.3	0.300	115.4									
	MDH	0.127	0.070	55.0	0.141	110.0	0.100	78.7	0.132	104.0	0.075	59.0	0.340	267									
	TP	350	250	71.4	320	91.4	400	114.2	275	78.6	200	57.0	240	68.5									
	SP	114	170	150.0	166	146.0	188	165.3	215	189.0	185	162.4	195	171.0									
	ISI	236	80	33.9	154	63.3	212	89.8	60	28.4	15	6.4	45	19.1									
	TPCHR	46.6	26.4	56.7	38.0	81.5	38.0	81.5	21.3	45.7	12.7	32.3	15.6	33.5									
	TAPAPS	26.6	10.4	40.0	15.6	58.6	19.0	71.4	14.3	54.0	6.3	27.0	10.2	38.5									
	TAPPS	20.0	16.0	80.0	22.4	112.0	19.0	95.0	7.0	35.0	6.4	32.0	5.4	27.0									
	TNPS	320	425	133.0	300	93.7	330	103.0	426	138.0	164	51.2	565	176.4									
	TL	37.3	28.4	76.0	24.4	65.3	84.8	227	639	171.1	69.7	186.6	40.3	107.8									
	ACHS	36.0	73.0	202	102	283	63.0	174.4	26.1	72.5	37.2	103.2	74.6	207									
T-ATPase	1.660	2.730	164.5	2.100	126.5	3.000	181.0	1.800	108.4	1.740	105.0	4.400	265										
Mg ²⁺ -ATPase	0.535	0.770	144.0	0.520	97.2	1.300	243	1.100	206	1.200	224	1.530	286										
Non-Mg ²⁺ -ATPase	1.125	1.960	1.740	1.580	157.5	0.520	130.0	1.730	433	1.000	250	1.500	375										
O	AAT	0.400	0.700	175.0	0.630	157.5	0.664	167.0	1.500	242	1.300	209	2.320	374									
	ALAT	0.621	1.322	213	0.672	108.2	0.200	47.2	0.053	12.5	0.305	72.0	0.620	146.2									
	SDH	0.424	0.108	25.5	0.160	37.7	0.020	4.0	0.020	8.0	0.240	95.2	0.110	44.6									
	LDH	0.252	0.119	47.2	0.200	80.0	0.175	70.0	0.020	8.0	0.300	83.3	0.350	97.2									
	GDH	0.360	0.104	28.8	0.200	55.5	0.182	50.5	0.071	20.0	0.305	82.3	0.140	93.0									
	MDH	0.151	0.140	93.0	0.110	73.0	0.261	106.6	0.070	46.3	0.205	135.3	0.140	93.0									
	Wet HSI	17.020	18.970	111.5	19.120	112.3	19.800	116.3	18.800	110.4	18.600	109.3	18.000	105.8									
	Dry HSI	8.760	9.212	105.2	10.522	120.1	9.300	106.1	10.570	120.7	7.700	87.9	8.000	91.3									
	WSI	8.250	9.760	118.3	8.600	104.2	10.500	127.2	8.900	100.6	10.900	132.1	10.031	121.6									
	TP	2534	2185	86.3	3137	123.8	2260	89.2	2715	107.1	2403	94.8	1964	77.5									
	SP	537	410	76.3	688	128.0	384	71.5	474	88.2	372	69.2	166	30.9									
	ISI	1997	1775	88.9	2449	122.6	1876	93.9	2241	112.2	2031	101.7	1798	90.0									
	TNPS	30.6	15.8	51.8	10.5	34.3	22.0	75.2	14.8	48.6	21.9	71.7	29.7	97.0									
	TL	533	983	184.4	1333	250	933	175.0	1450	272	1316	246	1766	330									
	TPCHR	102.2	61.7	60.4	89.3	87.4	73.8	72.2	87.3	85.4	107.7	105.4	54.6	53.4									
TAPAPS	69.0	32.7	47.4	47.6	69.0	42.9	62.3	51.7	75.0	85.5	124.0	37.3	54.0										
TAPPS	33.2	29.0	87.3	41.7	125.6	30.9	93.1	35.6	107.2	22.2	66.8	17.3	52.2										
O	Wet HSI	0.723	3.34	462	1.760	243	1.070	148.0	1.500	235	1.200	166.0	0.550	76.1									
	Dry HSI	0.300	1.421	474	0.890	297	0.500	166.7	0.734	178.0	0.620	206.7	0.160	53.3									
	WSI	0.425	1.920	452																			

CHAPTER VII 3

SOME PERCENTAGES

AND RATIOS

In the protein
pools the soluble
VII 3.A PROTEIN POOL and insoluble
(structural) pro-
tein fractions show different propor-
tional relations in different tissues.
These relations generally reflect the
metabolic and physiological status of
the tissues in question.

The stressant regimes appear to cause changes in these relations between tissue protein-fractions, signifying their deep influence on the metabolic status or 'personality' of the tissue.

In the hepatopancreas, gill and CTGM (cephalo-thoracic ganglionic mass) tissues of the crab, the soluble protein fraction is of considerably larger size than the insoluble protein fraction, whereas in the chelate leg muscle tissue the reverse holds good (Table VII 3.2).

TABLE VII 3.2: The proportions of soluble protein (SP) and insoluble protein (ISP) fractions of total protein (TP) pool in the tissues of O. senex senex.

Tissue	SP % TP	ISP % TP
HP	81.5	18.5
G	80.1	19.9
CTGM	80.0	20.0
M	32.6	67.4

Under the duress of the stressants, this 'balance' between the protein fractions of the total protein pools

is upset to different degrees in different regimes. In a general way, the SP-fraction shows decrement in the 'tissue group' including HP, G and CTGM and increment in the second tissue group comprising of the chelate leg muscle tissue (Tables VII 3.3, VII 3.4, VII 3.5 and VII 3.6).

TABLE VII 3.3: Changes in SP:ISP percentages in the hepatopancreas of O. senex senex under the different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TP	SP	ISP	SP % TP	ISP % TP
C		221	180	41	81.5	18.5
Cd	15 dps	420	218	202	51.1	48.9
Cd	30 dps	330	216	114	65.5	34.5
pH	15 dps	201	214	47	81.9	18.1
pH	30 dps	382	250	132	65.4	34.6
Comb	15 dps	275	216	59	78.5	21.5
Comb	30 dps	315	240	75	76.2	23.8
					

TABLE VII 3.4: Changes in SP:ISP percentages in the gill of O. senex senex under the different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TP	SP	ISP	SP % TP	ISP % TP
C		191	153	48	80.1	19.9
Cd	15 dps	250	171	79	68.4	31.6
Cd	30 dps	210	123	87	58.6	41.4
pH	15 dps	185	151	34	81.6	18.4
pH	30 dps	280	215	65	76.8	23.2
Comb	15 dps	246	198	48	80.5	19.5
Comb	30 dps	328	150	178	45.7	54.3

TABLE VII 3.5: Changes in SP:ISP percentages in the CTGM of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TP	SP	ISP	SP % TP	ISP % TP
C		150	120	30	80.0	20.0
Cd	15 dps	400	107	293	26.7	73.3
Cd	30 dps	202	110	92	54.4	45.6
pH	15 dps	300	162	138	54.0	46.0
pH	30 dps	260	230	30	88.5	11.5
Comb	15 dps	351	136	215	38.7	61.3
Comb	30 dps	280	250	30	89.3	10.7

TABLE VII 3.6: Changes in SP:ISP percentages in M of O. senex senex under the different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TP	SP	ISP	SP % TP	ISP % TP
C		350	114	236	32.6	67.4
Cd	15 dps	250	170	80	68.0	32.0
Cd	30 dps	320	166	154	51.8	48.2
pH	15 dps	400	188	212	47.0	53.0
pH	30 dps	275	215	60	78.8	21.2
Comb	15 dps	200	185	15	92.5	7.5
Comb	30 dps	240	95	45	81.2	18.8

The increase in the proportion of soluble protein pool of muscle is remarkable and is more significant beyond mere implication in the enhancement of activity levels of several enzyme systems. Under both Cd and pH duresses, a clear 'fluidization' of the chelate leg muscle has been noted similar to the phenomenology occurring in the ecdysial state of the crustaceans (Skinner, 1966) and

the local scorpion Heterometrus fulvipes (Raghavaiah et al., 1976). The comparison between the muscle of O. senex senex under stressant-duress and the 'ecdysial' muscle (vide ut supra) may however be not carried beyond the fact of fluidization. It has been noted elsewhere (Chapter VI) that the histosomatic index of muscle increases notably under toxicant stress in the present organism, whereas in the 'moulting' muscle of the crab, Gecarcinus lateralis (Skinner, 1966) and the scorpion H. fulvipes (Raghavaiah et al., 1976) considerable loss of muscle protein and decrement of HSI of muscle are noted.

Besides the solid tissues, the fluid tissue, haemolymph shows compositional alterations in the extra-cellular (ECP) and intracellular (ICP) protein fractions, under the duress of the stressants (Table VII 3.7).

TABLE VII 3.7: Changes in the ECP:ICP percentages in the haemolymph of O. senex senex under the different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TP	ECP	ICP	ECP % TP	ICP % TP
C		2534	537	1997	21.2	78.8
Cd	15 dps	2185	410	1775	18.8	81.2
Cd	30 dps	3137	688	2449	21.9	78.1
pH	15 dps	2260	384	1876	17.0	83.0
pH	30 dps	2714	474	2241	17.5	82.5
Comb	15 dps	2403	372	2031	15.5	84.5
Comb	30 dps	1964	166	1798	8.4	91.6

The proportion of ECP tends to decrease under the stressant regimes indicative of its participation to some extent in the biochemical economy (or non-economy ?) obtaining under the duress of the stressants.

In the present work, data for two fractions of the tissue carbohydrate pool are presented. One pool comprises of carbohydrate which precipitates along with protein when treated with the deproteinization agent, trichloroacetic acid (TCA). This fraction has been named total acid-precipitable anthrone-positive substances and acronymised TAPAPS.

VII 3.B CARBOHYDRATE
POOL

In the TCA-precipitation converse phase of the tissue viz., the TCA-extract, the second fraction of the carbohydrate pool is estimated. This fraction is called total acid-extractable anthrone-positive substances (TAEAPS). This latter fraction is homologous to the total anthrone-positive substances (TAPS)-pool of tissue often found in literature (Scheer, 1959; Raghavaiah and Ramamurthi, 1978; Raghavaiah et al., 1978). The rationale for inclusion of TAPAPS-fraction in the quantitation of tissues' total carbohydrate reserves (TCHR) is given elsewhere (Ramanaiah, 1978; Ramanaiah et al., 1982) and elaboration of the same will be extracontextual here.

In the 'normal' unstressed tissues of the crab, the TAEAPS fraction constitutes the more predominant constituent of the total carbohydrate reserves (TCHR) (Table VII 3.8).

TABLE VII 3.8: TAEAPS:TAPAPS percentages in the TCHR of 'normal' tissues of O. senex senex.

(Values computed from the data given in table VII 2.1).

Tissue	TAEAPS % TCHR	TAPAPS % TCHR
HP	74.4	25.6
G	62.3	37.7
CTGM	65.6	34.4
M	57.1	42.9
HL	67.5	32.5

Under the stressant regimes, the TAEAPS component is often subjected to diminution, so that its percentage status goes down, in the tissues. In the hepatopancreatic tissue, the TAEAPS percentage depression is felt under all regimes except the 30 dps combinational regime (Table VII 3.9).

TABLE VII 3.9: Changes in TAEAPS:TAPAPS percentages in hepatopancreas of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TCHR	TAEAPS	TAPAPS	TAEAPS % TCHR	TAPAPS % TCHR
C		39.1	29.1	10.0	74.4	25.6
Cd	15 dps	33.7	18.1	15.6	53.7	46.3
Cd	30 dps	28.7	10.4	18.3	36.2	63.8
pH	15 dps	26.2	12.6	13.6	48.1	51.9
pH	30 dps	27.1	16.0	11.1	59.0	41.0
Comb	15 dps	22.2	1.4	7.8	64.9	35.1
Comb	30 dps	38.0	32.0	6.0	84.2	15.8

In the gill tissue, the TAEAPS percentage depression is apparent under all the regimes except the 30 dps pH regime (Table VII 3.10).

TABLE VII 3.10: Changes in TAEAPS:TAPAPS percentages in gill of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	TCHR	TAEAPS	TAPAPS	TAEAPS % TCHR	TAPAPS % TCHR
C	40.9	25.5	15.4	62.3	37.7
Cd 15 dps	24.7	8.0	16.7	32.4	67.6
Cd 30 dps	30.5	17.5	13.0	57.4	42.6
pH 15 dps	35.0	14.3	20.7	40.9	59.1
pH 30 dps	23.5	15.0	8.5	63.8	36.2
Comb 15 dps	20.5	10.0	10.5	48.8	51.2
Comb 30 dps	16.5	8.2	8.3	49.7	50.3

In the cephalothoracic ganglionic mass (CTGM) of the crab, the TAEAPS percentage shows decrement of notable extent under shorter stress-duration regimes. Under longer stress-duration regimes, this percentage appears to be restored to normalcy (Table VII 3.11).

TABLE VII 3.11: Changes in TAEAPS:TAPAPS percentages in CTGM of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	TCHR	TAEAPS	TAPAPS	TAEAPS % TCHR	TAPAPS % TCHR
C	32.0	21.0	11.0	65.6	34.4
Cd 15 dps	29.3	12.0	17.3	41.0	59.0
Cd 30 dps	40.1	24.6	15.5	61.3	38.7
pH 15 dps	27.4	13.0	14.4	47.4	52.6
pH 30 dps	22.5	16.5	6.0	73.3	26.7
Comb 15 dps	30.5	10.5	20.0	34.4	65.6
Comb 30 dps	18.3	12.3	6.0	67.2	32.8

In muscle tissue also (Table VII 3.12), the TAEAPS percentage shows depression under shorter stress-duration regimes whereas an 'overshoot' of this percentage is generally evident under longer stress-duration regimes.

TABLE VII 3.12: Changes in TAEAPS:TAPAPS percentages in the chelate leg muscle of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	TCHR	TAEAPS	TAPAPS	TAEAPS % TCHR	TAPAPS % TCHR
C	46.6	26.6	20.0	57.1	42.9
Cd 15 dps	26.4	10.4	16.0	39.4	60.6
Cd 30 dps	38.0	15.6	22.4	41.1	58.9
pH 15 dps	38.0	19.0	19.0	50.0	50.0
pH 30 dps	21.3	14.3	7.0	67.1	32.9
Comb 15 dps	12.7	6.3	6.4	49.6	50.4
Comb 30 dps	15.6	10.2	5.4	65.4	34.6

In the haemolymph tissue of the crab, the TAEAPS percentage shows depression in individual regimes of Cd and pH and in the combinational regimes this percentage is elevated (Table VII 3.13).

TABLE VII 3.13: Changes in TAEAPS:TAPAPS percentages in haemolymph of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		TCHR	TAEAPS	TAPAPS	TAEAPS % TCHR	TAPAPS % TCHR
C		102.2	69.0	33.2	67.5	32.5
Cd	15 dps	61.7	32.7	29.0	53.0	47.0
Cd	30 dps	89.3	47.6	41.7	53.3	46.7
pH	15 dps	73.8	42.9	30.9	64.9	35.1
pH	30 dps	87.3	51.7	35.6	59.3	40.7
Comb	15 dps	107.7	85.5	22.2	79.4	20.6
Comb	30 dps	54.6	31.3	17.3	68.3	31.7

VII 3.C COMPONENTS OF ATPase SYSTEM

In the tissues of the crab, the components of ATPase system show percentage relation which reflects on the tissue personality or tissue specificity of metabolism (Table VII 3.14). In the hepatopancreas and chelate leg muscle tissues, the non-Mg²⁺-ATPase component of the ATPase system predominates

TABLE VII 3.14: Percentage composition of components of ATPase system in the tissues of O. senex senex.

(Values computed from the data given in table VII 2.1).

Tissue	Mg ²⁺ -ATPase % Total ATPase	Non-Mg ²⁺ -ATPase % Total ATPase
HP	44.2	55.8
G	65.0	35.0
CTGM	52.3	47.7
M	32.2	67.8

whereas in the gill and cephalothoracic ganglionic mass tissues, the Mg²⁺-ATPase component predominates. Between these two tissue-groups, there exist succinct differences in the energetic patterns if the percentage component composition of ATPase system is any indication.

Under the stressant duress, the percentage composition of Mg²⁺-ATPase component in the hepatopancreas tissue of the crab tends to be elevated, the exception being 30 dps combinational regime (Table VII 3.15).

TABLE VII 3.15: Changes in percentages of components of ATPase system of hepatopancreas of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	T.ATPase	Mg ²⁺ ATPase	Non- Mg ²⁺ ATPase	Mg ²⁺ ATPase	Non- Mg ²⁺ ATPase
				%	%
				T.ATPase	T.ATPase
C	1.620	0.717	0.903	44.2	55.8
Cd 15 dps	3.140	1.530	1.610	48.7	51.3
Cd 30 dps	1.040	0.563	0.477	54.1	45.9
pH 15 dps	1.700	1.200	0.500	70.6	29.4
pH 30 dps	0.800	0.453	0.347	56.6	43.4
Comb 15 dps	1.780	1.042	0.738	58.6	41.4
Comb 30 dps	4.010	1.300	2.719	32.4	67.6

In the gill tissue of the crab, under Cd-regime, the shorter stress-duration shows elevation of Mg²⁺-ATPase percentage (from 65.0% in normal state to 76.9% 15 dps) and the longer stress-duration, a depression (from 65.0% in normal state to 45.8% 30 dps) (Table VII 3.16). Under pH-regime, in the shorter stress-

TABLE VII 3.16: Changes in percentages of components of ATPase system of gill of O. senex senex under different Cd and pH regimes

(Values computed from the data given in table VII 2.1).

Stress	T.ATPase	Mg ²⁺ ATPase	Non- Mg ²⁺ ATPase	Mg ²⁺ ATPase %	Non- Mg ²⁺ ATPase %
				T.ATPase	T.ATPase
C	0.922	0.600	0.322	65.0	35.0
Cd 15 dps	0.550	0.423	0.127	76.9	23.1
Cd 30 dps	0.650	0.298	0.352	45.8	54.2
pH 15 dps	1.124	0.730	0.394	64.9	35.1
pH 30 dps	0.400	0.325	0.075	81.2	18.8
Comb 15 dps	1.000	0.611	0.389	61.1	38.9
Comb 30 dps	1.650	0.725	0.925	43.9	56.1

of
duration, a very small depression of Mg²⁺-ATPase percentage is evident, whereas in the longer stress-duration, a remarkable elevation of this percentage is noted (from 65% in normal state to 81.2% 30 dps). In the combinational regime, both durations of stress are associated with the

depression of Mg^{2+} -ATPase percentage (from 65% in normal state to 61.1% 15 dps and to 43.9% 30 dps).

TABLE VII 3.17: Changes in percentages of components of ATPase system of CTGM of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	T.ATPase	Mg^{2+} -ATPase	Non- Mg^{2+} -ATPase	Mg^{2+} -ATPase	Non- Mg^{2+} -ATPase
				% T.ATPase	% T.ATPase
C	0.254	0.133	0.121	52.3	47.7
Cd 15 dps	0.285	0.264	0.021	92.6	7.4
Cd 30 dps	0.183	0.122	0.061	66.6	33.4
pH 15 dps	0.350	0.330	0.020	94.2	5.8
pH 30 dps	0.185	0.126	0.059	68.1	31.9
Comb 15 dps	0.263	0.260	0.003	98.8	1.2
Comb 30 dps	1.301	0.614	0.687	47.2	52.8

In the cephalothoracic ganglionic mass of the crab, the Mg^{2+} -ATPase percentage (Table VII 3.17) is elevated in all regimes except 30 dps combinational regime. Especially, the elevations in the shorter stress-

durations of the regimes are astonishing (from 52.3% in normal state to 92.6% in Cd regime 15 dps; to 94.2% in pH regime 15 dps; to 98.8% in combinational regime 15 dps). The tissue's energetic state presumably undergoes remarkable shifts of a fundamental order under the stressant regimes.

TABLE VII.3.18: Changes in percentages of components of ATPase system of chelate leg muscle of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress	T.ATPase	Mg ²⁺ - ATPase	Non-	Mg ²⁺ -	Non-Mg ²⁺ -
			Mg ²⁺ -	ATPase	ATPase
			ATPase	%	%
				T.ATPase	T.ATPase
			- - - -	- - - -	- - - -
C	1.060	0.535	1.025	32.2	67.8
Cd 15 dps	2.730	0.770	1.960	28.2	71.8
Cd 30 dps	2.100	0.520	1.580	24.8	75.2
pH 15 dps	3.000	1.300	1.700	43.3	56.7
pH 30 dps	1.800	1.100	0.700	61.1	38.9
Comb 15 dps	1.740	1.200	0.540	68.9	31.1
Comb 30 dps	4.400	1.530	2.870	34.7	65.3

In the chelate leg muscle tissue too, the percentages of ATPase-fractions are altered under stressant regimes (Table VII 3.18). Under Cd-regime, the Mg^{2+} -ATPase percentage is depressed whereas under the pH- and combinational regimes, the percentage is elevated.

VII 3.D De RITIS QUOTIENTS

The De Ritis quotients computed for the aminotransferase activities of the different tissues of the crab show alterations under the duresses of the stressants (Table VII 3.19). In the cephalothoracic ganglionic mass, the quotient is subjected to elevation under Cd regime and depression under pH regime. In the combinational regime, in the shorter stress-duration, a remarkable elevation of the quotient is evident (from 1.5 in normal state to 3.113, 15 dps). In the longer stress-duration, the quotient is depressed.

In the chelate leg muscle, the quotient is generally elevated under the different stressant regimes.

In the hepatopancreas of the crab, the quotient is variably modified under the different stressant regimes. Under Cd regime, the modifications are of a small order. Under pH regime, the quotient is elevated

TABLE VII 3.19: De Ritis quotients of the aminotransferases (AAT, ALAT) in the tissues of O. senex senex under different Cd and pH regimes.

(Values computed from the data given in table VII 2.1).

Stress		De Ritis quotient for		
		CTGM	M	HP
C		1.500	0.644	1.527
Cd	15 dps	1.763	0.529	1.600
Cd	30 dps	2.221	0.937	1.560
pH	15 dps	0.492	0.783	1.826
pH	30 dps	1.192	1.153	1.071
Comb	15 dps	1.113	0.769	1.430
Comb	30 dps	1.337	0.646	1.363

in the shorter stress-duration and depressed in the longer stress-duration. Under combinational regime, in both stress durations, the quotient is depressed.

The variation in De Ritis quotient occurring in tissues of the crab under the duresses of the stressants underlines the point that the metabolism of

aminoacids in particular and nitrogenous metabolism in general are deeply influenced by the stressants.

Clearly the 'intergression points' between amino-acid metabolism and carbohydrate metabolism undergo 'shifts' under stressant duresses. One can discern aspects of stressant-specificity and tissue-specificity as well in the 'shifts' of 'metabolism intergression points'.

VII 3.E CHAPTERULAR
RESUME

(1) The percentages of the two fractions of protein pools, the soluble proteins (SP) and insoluble proteins (ISP) in the tissues of the crab, O. senex senex show alterations under the different regimes of Cd and pH. The SP-percentage is generally depressed under the different regimes in the tissues of the crab with the exception of chelate leg muscle. This tissue-specific protein compositional trait of chelate leg muscle has been assigned a causal role in the 'fluidization' of muscle under the stressant duresses.

(2) The percentage statuses of the fractions of total carbohydrate reserves (TCHR) of the tissues, the total acid-extractable anthrone-positive substances (TAEAPS) and total acid-precipitable anthrone-positive

substances (TAPAPS), undergo alterations in the tissues of O. senex senex under the different regimes of Cd and pH.

In general the percentage of TAPAPS is depressed in the tissues of the crab under the different stressant regimes indicative of the readiness with which this component of the TCHR participates in meeting the demands of tissues metabolism-in-duress.

(3) The components of the ATPase system, viz., Mg^{2+} -ATPase and non- Mg^{2+} -ATPases show alterations in their percentage composition in the tissues of O. senex senex under the different regimes of Cd and pH.

These alterations give evidence for stressant and tissue specificities. The remarkable elevations of Mg^{2+} -ATPase component of the ATPase system in the shorter stress-durations in CTGM illustrates the tissue-specificity aspect.

(4) The De Ritis quotients for the aminotransferase activities, show tissue-specific alterations in the tissues of O. senex senex under the stressant regimes. These alterations are considered to indicate the occurrence of 'shifts' of 'metabolism intergression-points' between carbohydrate and amino acid metabolisms in the tissues of the crab under the stressant duresses.

CHAPTERULE VII 4

HOLOHISTOMETRIC
PERSPECTIVE

The dataal retrospec-
tive provided in
VII 4.A 'KNOW-WHAT' table VII 2.1 in-
OF HOLO- cludes the levels of
HISTOMETRY bio-chemical consti-
tuents in tissues of the crab, O. senex
senex, under different stressant regimes,
expressed in relation to the weight of
the tissue ('weight-specific levels').

Similarly, the table cited, gives data on the activity levels of enzymes in tissues, expressed into units of soluble protein of the tissues in question ('soluble protein-specific levels').

But then the tissues of the crab have been found to undergo alterations in their weight statuses, variably, under the different stressant regimes studied. Reasonably this aspect has to be put in perspective while evaluating the effect of the stressants on the levels of biochemical constituents. Implication of the tissue weight status gives an additional elucidational dimension to the action of the stressants on the tissue biochemistry.

Alteration of tissue weight under the duresses of stressants logically involves alterations in the size of the protein pool and soluble-protein component thereof. This point has to be given due consideration while evaluating the influence of the toxicants on the tissue enzyme-activity statuses. Thus the weight of the tissue gives one more dimension (or 'sub-dimension'?) for evaluation of stressant 'toxicophany'.

This approach involving the tissue weight has been found useful in several stress-situations in which organisms are placed. Especially when the stressants cause conspicuous alterations in somatic and histontic

(individual tissue) weight statuses, implication of this weight dimension appears to be imperative for obtaining a fuller insight into the influence of the toxicant on the organismic and tissue biology.

In this crab, O. senex senex this approach has been used to illustrate salinity stress and its influence on organic and tissue biology (Venkata Reddy, 1976). In the amphibious apple snail, Pila globosa, dramatic somatic and tissue weight reductions are found during induced aestival torpor stress and in this situation also implication of weight dimension has formed a useful elucidational tool in interpretation of aestivation induced tissue biochemistry and enzymo-metry (Chandrasekharam, 1977).

The approach is called holohistometric (total tissue measurement) approach, since this involves measurement or calculation of weight-specific levels of biochemical constituents of a tissue into the total weight of the tissue.

The approach of holohistometry is essentially calculational. The histosomatic indices obtained for the

VII 4.B 'DO-HOW' OF HOLOHISTOMETRY

different tissues are used for this purpose.

The weight specific level (WSL) of a biochemical constituent when multiplied with the histosomatic index (HSI) of the tissue will yield the quantum of the constituent in the total tissue in 100 gram-heavy organism (i).

$$\text{WSL} \cdot \text{HSI} = \text{HHL} \quad \dots \quad (i)$$

This quantum is named holohistontic level (HHL) of the constituent.

For computation of the HHL of enzyme activities, the HHL of soluble protein (SP) of the tissue is employed as the multiplication factor. The soluble protein-specific level of enzyme activity (SPSL) multiplied into the holohistontic level of soluble protein (HHLSP) gives the holohistontic enzyme level (HHLE) (ii).

$$\text{SPSL} \cdot \text{HHLSP} = \text{HHLE} \quad \dots \quad (ii)$$

Using the 'do-how' for holohistometry given above

VII 4.C HOLOHISTOMETRY (VII 4.B) the data of VII 2.1
IN CRAB UNDER are calculated into the total
STRESSANT DURESS tissue weights in the crab,

O. senex senex under the different stressant regimes.

Tables VII 4.20 to VII 4.22 provide the HHLs for the biochemical constituents in the tissues of the crab.

Tables VII 4.23 to VII 4.25 give the holohistontic enzyme

levels in the different tissues of the crab under the different stressant regimes. Table VII 4.26 gives data for haemolymph for which an assumed haemolymph volume index (HLVI) is employed for arriving at total haemolymph levels of the organic constituents. The quanta and percentage changes obtained from holohistometric approach may be compared with the data given in table VII 2.1 providing the weight-specific estimations of organic constituents and soluble protein-specific levels of the activities of enzymes.

In hepatopancreas, the constituents that undergo reduction in the weight specific expression, show an accentuation of this change under 'holohistometric view'. The constituent that show elevations in their weight specific expressions, show 'de-elevation' of the change under holohistometric view. This is due to the fact that this tissue undergoes reduction in weight under different stressant regimes, to different degrees.

In the chelate leg muscle tissue, which undergoes weight-increase under the different stressant regimes, a reversed situation is obtainable: i.e., holohistometric accentuation of increased weight-specific levels of the biochemical constituents and holohistometric de-depression of decreased weight-specific levels.

TABLE VII 4.20:

Holohistontic levels (HHL) of organic constituents in hepatopancreas of O. senex senex under different regimes of Cd and pH.

(HSIs used in computation of HHL : C : 8.012; Cd 15 dps : 3.65;

Cd 30 dps : 5.80; pH 15 dps : 4.54; pH 30 dps : 4.9; Comb 15 dps : 5.007;

Comb 30 dps : 4.210).

Parameter	Control (I _{as})	Stress	15 dps			30 dps		
			1 _{ps}	ps % as	Change ps % as	1 _{ps}	ps % as	Change ps % as
1	2	3	4	5	6	7	8	9
TP	1171	Cd	1533	86.6	- 13.4	1914	108.1	+ 8.1
		pH	1185	66.9	- 33.1	1872	105.7	+ 5.7
		Comb	1377	77.8	- 22.2	1326	74.5	- 25.5
SP	1442	Cd	796	55.2	- 44.8	1253	86.9	- 13.1
		pH	972	67.4	- 32.6	1225	85.0	- 15.0
		Comb	1082	75.0	- 25.0	1010	70.0	- 30.0
ISP	328	Cd	737	225	+ 125.0	638	194.5	+ 94.5
		pH	213	64.9	- 35.1	647	197.3	+ 97.3
		Comb	295	89.9	- 10.1	314	95.7	- 4.3

(CONTD. TABLE VII 4.20)

1	2	3	4	5	6	7	8	9
TNPS	1883	Cd	1048	55.7	- 44.3	1456	77.3	- 22.7
		pH	3087	163.9	+ 63.9	1490	79.1	- 20.9
		Comb	1051	55.8	- 44.2	1587	84.3	- 15.7
TL	897	Cd	569	63.4	- 36.6	696	77.6	- 22.4
		pH	817	91.1	- 8.9	701	78.1	- 21.9
		Comb	781	87.1	- 12.9	493	55.0	- 45.0
TCHK	313	Cd	123	39.3	- 60.7	166.4	53.2	- 46.8
		pH	118.9	38.0	- 62.0	132.8	42.4	- 57.6
		Comb	111.2	35.5	- 64.5	160.0	51.1	- 48.9
TAEAPS	233	Cd	66	28.3	- 71.7	60.3	25.9	- 74.1
		pH	57.2	24.5	- 75.5	78.4	33.6	- 66.4
		Comb	72.1	30.9	- 69.1	135	57.9	- 42.1
TAPAPS	80.1	Cd	56.9	71.0	- 29.0	106.1	132.5	+ 32.5
		pH	61.7	77.0	- 23.0	54.4	67.9	- 32.1
		Comb	39.1	48.8	- 51.2	25.0	31.2	- 68.8

TABLE VII 4.21: Holohistontic levels (HHL) of organic constituents in gill of O. senex
senex under different regimes of Cd and pH

(MSIs used in computation of HHL : C : 1.430; Cd 15 dps : 1.343;
Cd 30 dps : 2.500; pH 15 dps : 1.550; pH 30 dps : 2.600; Comb 15 dps :
1.560; Comb 30 dps : 1.415).

Parameter	Control (I _{as})	Stress	15 dps			30 dps		
			I _{ps} 4	ps % as 5	Change ps % as 5	I _{ps} 7	ps % as 8	Change ps % as 9
TP	273	Cd	336	123.1	+ 22.1	525	192.3	+ 92.3
		pH	287	105.1	+ 5.1	728	267.0	+ 167
		Comb	384	140.7	+ 40.7	464	170.0	+ 70.0
SP	219	Cd	230	105.0	+ 5.0	308	140.6	+ 40.6
		pH	234	106.9	+ 6.9	559	255.0	+ 155
		Comb	309	141.1	+ 41.1	212	96.8	- 3.2
ISP	54	Cd	106	196.3	+ 96.3	218	404	+ 30.4
		pH	53	98.1	- 1.9	169	313	+ 213
		Comb	75	138.9	+ 38.9	252	467	+ 367

(CONTD. TABLE VII 4.21)

1	2	3	4	5	6	7	8	9
TNPS	429	Cd	488	113.8	+ 13.8	820	191.1	+ 91.1
		pH	744	173.4	+ 73.4	798	186.0	+ 86.6
		Comb	491	114.5	+ 14.5	533	124.2	+ 24.2
TL	95.5	Cd	66.1	69.2	- 30.8	151	158.0	+ 58.0
		pH	27.2	28.5	- 71.5	95.2	99.7	- 0.3
		Comb	151	158.0	+ 58.0	137	143.5	+ 43.5
TCHR	58.5	Cd	33.1	56.6	- 43.4	76.3	130.4	+ 30.4
		pH	54.3	92.8	- 7.2	61.1	104.4	+ 4.4
		Comb	32.0	54.7	- 45.3	23.3	39.8	- 60.2
TAEAPS	36.5	Cd	10.7	29.3	- 70.7	43.8	120.0	+ 20.0
		pH	22.2	60.8	- 39.2	39.0	106.8	+ 6.8
		Comb	15.6	42.7	- 57.3	11.6	31.8	- 68.2
TAPAPS	22.0	Cd	22.4	101.8	+ 1.8	32.5	147.7	+ 47.7
		pH	32.1	145.9	+ 45.9	22.1	100.5	+ 0.5
		Comb	16.4	72.7	- 27.3	11.7	53.2	- 46.8

TABLE VII 4.22: Holohistontic levels (HHL) of organic constituents in chelate leg muscle of O. senex senex under different regimes of Cd and pH.

(HSIs used in computation of HHL: C : 17.020; Cd 15 dps: 18.970;

Cd 30 dps: 19.120; pH 15 dps: 19.800; pH 30 dps: 18.800; Comb 15 dps :

18.600; Comb : 30 dps : 18.000).

Parameter	Control (1 _{as})	Stress	15 dps			30 dps		
			l _{ps}	ps % as	Change ps % as	l _{ps}	Ps % as	change ps % as
1	2	3	4	5	6	7	8	9
TP	5957	Cd	4743	79.6	- 20.4	6118	102.7	+ 2.7
		pH	7920	133.0	+ 33.0	5170	86.8	- 13.2
		Comb	3720	62.4	- 37.6	4320	72.5	- 27.5
SP	1940	Cd	3225	166.2	+ 66.2	3174	163.6	+ 63.6
		pH	3722	191.9	+ 91.9	4042	208.0	+ 108.0
		Comb	3441	177.4	+ 77.4	3510	180.9	+ 80.9
ISP	4017	Cd	1518	37.8	- 62.2	2944	73.3	- 26.7
		pH	4198	104.5	+ 4.5	1128	28.1	- 71.9
		Comb	279	7.0	- 93.0	810	20.2	- 79.8

TNPS	5446	Cd	8062	148.0	+ 48.0	5736	105.3	+ 5.3
		pH	6534	120.0	+ 20.0	8009	147.1	+ 47.1
		Comb	3050	56.0	- 44.0	10170	186.7	+ 86.1
TL	635	Cd	539	84.9	- 15.1	467	73.5	- 26.5
		pH	1679	264	+ 164	1201	189.1	+ 89.1
		Comb	1296	204	+ 104	725	114.2	+ 14.2
TCHR	793	Cd	501	63.2	- 36.8	726	91.6	- 8.4
		pH	752	94.8	- 5.2	401	50.6	- 49.4
		Comb	236	29.8	- 70.2	281	35.4	- 64.6
TAPPS	453	Cd	197	43.5	- 56.5	298	65.8	- 34.2
		pH	376	83.0	- 17.0	269	59.4	- 40.6
		Comb	117	25.8	- 74.2	184	40.6	- 59.4
TAPPS	340	Cd	304	89.4	- 10.6	428	125.9	+ 25.9
		pH	376	110.6	+ 10.6	132	38.8	- 61.2
		Comb	119	35.0	- 65.0	97	28.5	- 71.5

TABLE VII 4.23: Holohistontic levels (HHL) of enzyme activities in hepatopancreas of O. senex senex under different regimes of Cd and pH.

(Holchistontic soluble protein levels (in mg) used in computation of
HHL: C: 1442; Cd 15 dps: 796; Cd 30 dps: 1253; pH 15 dps: 972;
pH 30 dps: 1225; Comb 15 dps : 1082; Comb 30 dps : 1010).

Parameter	Control (1 _{as})	Stress	15 dps			30 dps		
			1 _{ps}	ps % as	Change ps % as	1 _{ps}	ps % as	Change ps % as
1	2	3	4	5	6	7	8	9
AAT		Cd	3184	256.0	+ 156	1291	103.8	+ 3.8
	1244	pH	887	71.3	- 28.7	3675	295.0	+ 195
		Comb	1547	124.4	+ 24.4	3030	244.0	+ 144
ALAT		Cd	1990	244.0	+ 144	827	101.5	+ 1.5
	815	pH	486	59.6	- 40.4	3430	421.0	+ 321
		Comb	1082	132.8	+ 32.8	2222	273.0	+ 173
T ₁ ATPase		Cd	2499	107.0	+ 7.0	1303	55.8	- 44.2
	2336	pH	1652	70.7	- 29.3	980	42.0	- 58.0
		Comb	1926	82.4	- 17.6	4050	173.3	+ 73.3

(CC TD TABLE VII 4.23

1	2	3	4	5	6	7	8
Mg ²⁺ -ATPase	1034	Cd	1218	117.8	+ 17.8	705	68.2
		pH	1166	112.8	+ 12.8	555	53.7
		Comb	1127	109.0	+ 9.0	1313	127.0
Non-Mg ²⁺ -ATPase	1302	Cd	1281	98.4	- 1.6	598	45.9
		pH	486	37.3	- 52.7	425	32.6
		Comb	799	61.4	- 38.6	2737	210.0
SDH	397	Cd	1218	307	+207	834	210
		pH	324	81.6	- 18.4	523	131.7
		Comb	288	72.5	- 27.5	303	76.3
LDH	174	Cd	460	264	+ 164	301	173.0
		pH	126	72.4	- 27.6	53.9	31.0
		Comb	141	81.0	- 19.0	202	116.1
GDH	193	Cd	417	216	+ 116	259	134.2
		pH	104	53.9	- 46.1	123	63.7
		Comb	128	66.3	- 33.7	235	121.8
MDH	361	Cd	517	143.2	+ 43.2	419	116.1
		pH	139	38.5	- 61.5	147	40.7
		Comb	110	30.5	- 69.5	884	245

TABLE VII 4.24: Holohistontic levels (HHL) of enzyme activities in gill of O. senex senex under different regimes of Cd and pH.

(Holohistontic soluble protein levels (in mg) used in the computation of HHL: C: 219; Cd 15 dps: 230, Cd 30 dps: 308; pH 15 dps: 234; pH 30 dps: 559; Comb 15 dps: 309; Comb 30 dps: 212).

Parameter	Control (1 as)	Stress	15 dps			30 dps		
			l ps	ps % as	Change ps % as	l ps	ps % as	Change ps % as
T-ATPase	202	Cd	127	62.9	- 37.1	200	99.0	- 1.0
		pH	263	130.2	+ 30.2	224	110.9	+ 10.9
		Comb	309	153.0	+ 53.0	350	173.3	+ 73.3
Mg ²⁺ -ATPase	131	Cd	97	74.0	- 26.0	92	70.2	- 29.8
		pH	171	130.5	+ 30.5	182	138.9	+ 38.9
		Comb	189	144.3	+ 44.3	154	117.6	+ 17.6
Non-Mg ²⁺ -ATPase	71	Cd	30	42.3	- 57.7	108	152.1	+ 52.1
		pH	92	129.6	+ 29.6	42	59.2	- 40.8
		Comb	120	169.0	+ 69.0	196	276.0	+ 176

(CONTD. TABLE VII 4.24)

1	2	3	4	5	6	7	8	9
SDH	215	Cd	118	54.9	- 45.1	105	48.8	- 51.2
		pH	234	108.8	+ 8.8	372	173.0	+ 73.0
		Comb	169.4	78.6	- 21.4	31.8	14.8	- 85.2
LDH	60.0	Cd	30.8	51.3	- 48.7	33.0	55.0	- 45.0
		pH	20.1	33.5	- 66.5	57.6	96.0	- 4.0
		Comb	47.9	79.8	- 20.2	6.4	10.7	- 89.3
GDH	137	Cd	69.5	50.7	- 49.3	77.0	56.2	- 43.8
		pH	70.7	51.6	- 48.4	341	249	+ 149
		Comb	144	105.1	+ 5.1	4.9	3.6	- 96.4
MDH	77.3	Cd	90.6	117.2	+ 17.2	85.3	110	+ 10.3
		pH	79.6	103.0	+ 3.0	224	290	+ 190
		Comb	35.9	46.4	- 53.6	19.1	24.7	- 75.3

TABLE VII 4.25: Holohistontic levels (HHL) of enzyme activities in the chelate leg muscle of O. senex senex under different regimes of Cd and pH

(Holohistontic soluble protein levels (in mg) used in computation of HHL:
 C: 1940; Cd 15 dps: 3225; Cd 30 dps: 3174; pH 15 dps: 3722; pH 30 dps:
 4042; Comb 15 dps: 3441; Comb 30 dps: 3510).

Parameter	Control (l_{as})	Stress	15 dps			30 dps		
			l_{ps}	ps % as	Change ps % as	l_{ps}	ps % as	Change ps % as
1	2	3	4	5	6	7	8	9
AAT	776	Cd	2258	291.0	+ 191	2000	258.0	+ 158
		pH	1935	249.0	+ 149	6993	901.0	+ 801
		Comb	3441	443.0	+ 343	3441	443.0	+ 343
ALAT	1205	Cd	4263	354.0	+ 254	2133	177.0	+ 77.0
		pH	2471	205.0	+ 105	6063	503.0	+ 403
		Comb	4473	371.0	+ 271	8143	676.0	+ 576
T-ATPase	3220	Cd	8804	273.0	+ 273	6665	207.0	+ 107
		pH	11166	347.0	+ 247	7276	226.0	+ 126
		Comb	5987	185.9	+ 85.9	15444	480.0	+ 380
Mg ²⁺ -ATPase	1038	Cd	2483	239.0	+ 139	1650	159.0	+ 59.0
		pH	4839	466.0	+ 366	4446	428.0	+ 328
		Comb	4130	398.0	+ 298	5370	517.0	+ 417

(CONTD. TABLE VI, 4.25)

1	2	3	4	5	6	7	8	9
Non-Mg ²⁺ - ATPase	2182	Cd	6321	290.0	+ 190	5015	230.0	+ 130
		pH	6327	290.0	+ 190	2830	129.7	+ 29.7
		Comb	1857	85.1	- 14.9	10074	462.0	+ 362
SDH	823	Cd	348	42.3	- 57.7	508	61.7	- 38.3
		pH	744	90.4	- 9.6	214	26.0	- 74.0
		Comb	1050	127.6	+ 27.6	2176	264	+ 164
LDH	489	Cd	384	78.5	- 21.5	635	129.9	+ 29.9
		pH	651	133.1	+ 33.1	80.8	16.5	- 83.5
		Comb	826	168.9	+ 68.9	386	78.9	- 21.1
GDH	698	Cd	335	48.0	- 52.0	635	91.0	- 9.0
		pH	677	97.0	- 3.0	287	41.1	- 58.9
		Comb	1032	147.9	+ 47.9	1229	176.1	+ 76.1
MDH	293	Cd	452	154.3	+ 54.3	349	119.1	+ 19.1
		pH	599	204	+ 104	283	96.6	- 3.4
		Comb	705	241	+ 141	491	167.6	+ 67.6
AChE	69840	Cd	235425	337	+ 237	323748	464	+ 364
		pH	234486	336	+ 236	105496	151.1	+ 51.1
		Comb	128905	183.0	+ 83.0	261846	375	+ 275

TABLE VII 4.26: Holohistontic levels (HHL) of organic constituents in haemolymph of O. senex senex under different regimes of Cd and pH.

(For computation of HHL, a value of 25.0% has been used as the haemolymph volume for all experimental situations. This value is obtained in normal crab (Bharani Kumar, unpublished observation, 1985).

Parameter	Control (l _{as})	Stress	15 dps			30 dps		
			l _{ps}	ps % as	change ps % as	l _{ps}	ps % as	Change ps % as
1	2	3	4	5	6	7	8	9
TP	634	Cd	546	86.1	- 13.9	784	123.7	+ 23.7
		pH	565	89.1	- 10.1	679	107.1	+ 7.1
		Comb	601	94.8	- 5.2	491	77.4	- 22.6
ECP	134	Cd	103	76.9	- 23.1	172	128.4	+ 28.4
		pH	96	71.6	- 28.4	118	88.1	- 11.9
		Comb	93	69.4	- 30.6	41	30.6	- 69.4
ICP	500	Cd	443	88.6	- 11.4	114	22.8	- 77.2
		pH	469	93.8	- 5.2	561	112.2	+ 12.2
		Comb	508	101.6	+ 1.6	450	90.0	- 10.0
TL	133	Cd	246	185.0	+ 85.0	333	250	+ 150
		pH	233	175.2	+ 75.2	362	272	+ 172
		Comb	329	247	+ 147	442	332	+ 232

(CONTD. TABLE VII 4.26)

1	2	3	4	5	6	7	8	9
TNPS	7.6	Cd pH Comb	3.9 6.0 5.5	51.3 78.9 72.4	- 48.7 - 21.1 - 27.6	2.6 3.7 7.4	34.2 48.7 97.4	- 65.8 - 51.3 - 2.6
TCHR	25.6	Cd pH Comb	15.4 18.4 27.0	60.2 71.9 105.5	- 39.8 - 28.1 + 5.5	22.3 21.8 23.6	87.1 85.2 92.2	- 12.9 - 14.8 - 7.8
TAEAPS	17.3	Cd pH Comb	8.1 10.7 21.4	46.8 61.8 123.7	- 53.2 - 38.2 + 23.7	11.9 12.9 9.3	68.8 74.6 53.8	- 31.2 - 25.4 - 46.2
TAPAPS	8.3	Cd pH Comb	7.3 7.7 5.6	88.0 92.8 67.5	- 12.0 - 7.2 - 32.5	10.4 8.9 4.3	125.3 107.2 51.8	+ 25.3 + 7.2 - 48.2

Since the holohistometric perspective takes into consideration the 'totality of the tissue', it gives a more lucid picture of the levels of reserves — biochemical and biocatalytic — in the tissues of the organism under the duress in question. Thus this paves the way for a 'holoscopy' of the organismic biochemistry, facilitating vivid visualization of inter-tissue metabolic and metabolite transactions.

One reading that can be made into the data holohistometrically derived is about the trends of change in the organic and enzymic (activity) composition of the tissue under different stressant regimes along exposure timescale. The data for changes in the levels of constituents noted at two exposure (stress) durations appear to fall into certain trendic categories and this categorisation is termed 'trendography'. The results of the trendographic exercise are given in fig. VII 4.1 and table VII 4.27. For the cephalothoracic ganglionic mass of the crab, holohistometry has not been carried out. Therefore, for this tissue, trends have been visualised for the weight specific levels (of organic components) and soluble protein-specific levels (of the enzyme activities) (Table VII 4.28).

In all, 10 trend-categories have been identified whose profiles are given in fig. VII 4.1. The first trend-category pertains to a positive change in the level/.

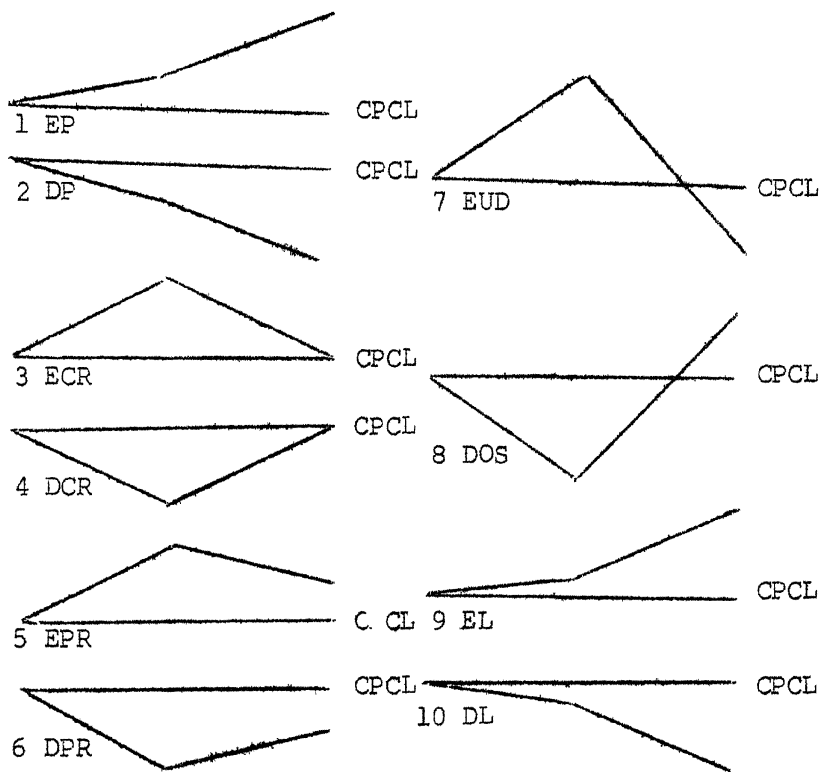


FIG. VII 4.1 TRENDGRAMS: The acronyms of the trendograms are expanded in the text. CPCL represents 100% (centum per centum) line.

activity of a component/activity which is progressive. Elevation 15 dps followed by still greater elevation 30 dps, is considered to belong to this category (Progressive elevation, EP). The converse of this theme is considered as the second

trend-category (Progressive depression, DP). The third trend-category describes a positive (elevatory) change 15 dps, which is followed by almost nil change 30 dps (Elevation; complete recovery, ECR). The fourth trend-category describes the changes which are the converse of changes under trend-category three (Depression; complete recovery, DCR). The fifth and sixth trend-categories pertain to changes 15 dps which return to normality, 30 dps, only partially. Trend-category five involves elevation (15 dps) and partial recovery (30 dps) (EPR). Trend-category six involves depression (15 dps) and partial recovery (30 dps) (DPR). In the assessment of recovery, 30 dps, a margin of 5% about the normal (= control, 100%) value is taken as zone of no change.

In trend-category seven the 15-dps-elevation is followed by notable depression, 30 dps so much so that the trend-line dips under the normal line (Elevation, under-dip, EUD). Trend-category eight describes changes which are converse of the trend-category seven. In this, the 15 dps depression, is followed by elevation 30 dps so that the trend line 'shoots' over the normal line (Depression, overshoot, DOS).

In trend-category nine, the level/activity shows no change 15 dps (see above about the 'zone of no change'). At the longer stress-duration, an elevation is recorded

and this 'late' elevation is included in trend-category nine (EL). In trend-category ten, nil-change 15 dps is followed by a good depression at the later (longer) stress-duration (~~Depression~~ late, DL).

In the trends of changes of protein pools of the tissues, elevation (E) happen to be the frequent trendic component for gill, chelate leg muscle and cephalothoracic ganglionic mass of the crab (O. senex senex). This trendic composition is shared by the three stressant regimes viz., Cd (in severo regime), pH (in severo regime) and the combinational (in combinatio regime).

The effect of the stressants on the protein pools and therefore on the protein metabolism of these tissues, should be obvious.

In the hepatopancreas and haemolymph on the other hand, depression appears as the greatest common trendic component. Thus in these tissues, the protein-pool depression, seems to be 'ordered' by the stressants under their metabolic presidency.

One has to recognize, therefore, in the sub-somatic stage of the organism two opposite physiological/biochemical propensities: One led by hepatopancreas and the other led by the chelate leg muscle.

Examination of trendographic pictures of the other biochemical/enzymic facets of the tissues vis-a-vis the stressant regimes will corroborate the propensities noted above.

The levels of TNPS in hepatopancreas and haemolymph are characterized by the 'depressive' trendic component as against the 'elevatory' trendic component noted in gill, chelate leg muscle and cephalothoracic ganglionic mass.

With regard to the total lipid pool of the tissues, the situation become a bit different. In this case, chelate leg muscle and haemolymph come to show elevation as the common trendic components. The other tissues, viz., hepatopancreas, gill and cephalothoracic ganglionic mass show depression as the predominant trendic component.

These lipid data trends serve to highlight the role of haemolymph in lipid transport under stress (or, is it the role of haemolymph lipid in stressant detoxification and transport, a la metallothioneins ?) and the participation of the component in the metabolic milieu of muscle under the stressant duress.

In the carbohydrate context, the tissue-specific physiological propensities visualized above become un-

palpable. All the tissues appear to be overwhelmed by depression as the trendic component. This observation underlines the point that the carbohydrate pools of the tissues are almost 'ritualistically' influenced (in the depression direction) by the stressant regimes. This trend of 'carbohydrate' consumption appears to be shared by the toxicants irrespective of the chemical functional taxonomy (Venkata Reddy, 1976; Bhagyalakshmi, 1981 and Balavenkatasubbaiah, 1985). This being the situation with regard to the total carbohydrate reserves (TCHR) of the tissues, the cephalothoracic ganglionic mass shows a queer trend of elevation of TAPAPS fraction of TCHR (Table VII 4.28) as against depression of this component noted in the other tissues. Does it reflect any tissue specific compensatory or 'reservatory' mechanism ?

The close involvement of TAPAPS fraction of TCHR is evident in all the tissues including CTGM. This indicates that under the metabolic presidency of the stressants, the TAPAPS-kinetic elements of metabolism are also influenced. Earlier, the TAPAPS-kinetic machinery has been shown to be responsive to neuroendocrine principles in invertebrates like Laevicaulis alte (Ramanaiiah et al., 1982). Now, we have evidence for the mechanism being 'responsive' to the stressant materials! It is possible that the TAPAPS-kinesis under the aegis of the stressants

is mediated by neuroendocrine principles. Evidence is available for pesticide-superintendence on hyperglycaemia caused by neuroendocrine principles in crab (Bhagyalakshmi et al., 1982).

The TAEAPS corresponds to the total anthrone-positive substances (TAPS), profusely reported in literature (Mc Whinnie & Scheer, 1958; Meenakshi & Scheer, 1961; Nowosielski & Patton, 1964; Dean & Vernberg, 1965; Ramamurthi, 1968; Ramamurthi & Veerabhadrachari, 1975; Vijayalakshmi & Kurup, 1976; Raghavaiah et al., 1978; Subramanyam, 1981; Pavankumar et al., 1982; Subramanyam & Ramamurthi, 1982a; Subramanyam & Ramamurthi, 1982b) and the kinetic mechanisms of this component of TCHR, named classically 'glycolytic machinery' are too well known to be elaborated here. Literature insights into the influence of stressants on this 'TAPS-kinetic' machinery are also available aplenty (Kleinholtz et al., 1950; Steele, 1963; Kulkarni, 1975).

In the trendographic picture of enzyme activities in tissues of O. senex senex under the stressant regimes, the 'multistar' elevation-phenomenology noted for the aminotransferases deserves a special mention. The assignment of star is only an arbitrary approach without any literature parallel or corroboration. However, this approach has been found convenient to

give a 'very special status' tag to this catalytic macromolecular system located at the 'metabolic-intergression points'. About the metabolic-intergression points and the aminotransferase system an elaborate mention is made else-where (see VII 3.D).

The ATPase system of hepatopaneas is generally depressed, while one component of the system viz., Mg^{2+} -ATPase is consistently elevated. Non- Mg^{2+} -ATPase component of the system is consistently depressed. In this respect, hepatopaneas resembles cephalothoracic ganglionic mass (CTGM) (Table VII 4.28). While no significance can be attached to the depression of non- Mg^{2+} -ATPase component in hepatopaneas, in CTGM, the depression of this component may have profound physiological significance.

In muscle, both components of the ATPase system are significantly elevated and in the combinational regime, the non- Mg^{2+} -ATPase component shows a four-star elevation ! The functional significance of this allround elevation of activity of ATPase system should at once be apparent. For, such elevations will lead to depletion of the energy charge of the tissue, with serious, long-term implications in the histobiology of muscle under stressant duress.

All the dehydrogenases in the tissues share depression as the greatest common trendic component except chelate leg muscle MDH (Table VII 4.27). This connotes the 'gloom' of depression which takes over the dehydrogenase systems of the tissues and the depression of energetic status. This scenario accords well with the ATP-lysis prodigality indicated by elevated ATPase activity noted above. This picture of energetic gloom under the aegis of stressants agrees with the work of Kennicutt (1980) who finds depletion of ATP reserves as the most conspicuous (and, deleterious) impact of the stressants and toxins on the biological systems.

The trendic profiles for dehydrogenases of the tissues of the crab, are characterised by depression as the predominant trendic component with the negative energetic implication emerging therefrom. One may add this to the 'ATP-frittering profile' noted above to get a full picture of the serious 'energetic crisis', which underlies the apparent 'sublethal' nature of the stressant regime!

Amidst this depressed dehydrogenase situation, the elevatory profile of muscular MDH stands out. What does the consistent elevation of MDH in muscle under the different regimes of Cd and pH indicate? A compensatory mechanism? These queries find no immediate solutions.

The activity levels of AChE have been found to be elevated consistently in chelate leg muscle (Table VII 4.27) and a bit less consistently in the cephalothoracic ganglionic mass of the crab.

The immediate interpretational implication of this AChE-elevation is in the elevation of tissue excitability. And this physiological escalation in turn may contribute to the 'energetic drain' visualized above and make the metabolic strain on the organism still seriousness.

VII 4.E CHAPTERULAR RE'SUME'

- a) The weight-specific levels
of organic constituents,
and soluble protein-specific
levels of enzyme activities

of tissues of O. senex senex under different Cd and pH regimes obtain additional illustrative, elucidational dimension when holohistometric is applied to them.

- b) Trendograms obtained from holohistometry illustrate the changes of the constituents and enzymes as a function of stress-duration.

- c) Interesting trendic pictures are obtained for the protein pools of the tissues, with elevational emphasis. This elevational emphasis is appreciable in three tissues viz., gill, chelate leg muscle and cephalo-

thoracic ganglionic mass. On the other hand, the trendic profiles of hepatopaneas and haemolymph are characterised by depression as the most common trendic component.

d) The levels of TNPS, which include free amino acids show depressional 'trend'-ency in hepatopaneas and haemolymph and elevatory 'trend'-ency in gill, chelate leg muscle and cephalothoracic ganglionic mass.

e) The lipid pools of chelate leg muscle and haemolymph are elevated under the stressant regimes and those of hepatopaneas, gill and cephalothoracic ganglionic mass are depressed.

f) The carbohydrate pools are generally depressed in the tissues of the crab under the stressant regimes.

g) The levels of aminotransferases of tissues are subjected to 'multi-starred' elevation under the stressant regimes.

h) The ATPase system is diversly influenced in the different tissues of the crab under the stressant regimes. In the cephalothoracic ganglionic mass and hepatopaneas, non-Mg²⁺-ATPase component of ATPase system is depressed.

In muscle, the ATPase system is subjected to remarkable elevation and the non-Mg²⁺-ATPase component shows four-star elevation.

i) The levels of dehydrogenases are characterised by depression as the most common trendic component.

j) The levels of AChE activity are elevated in chelate leg muscle and cephalothoracic ganglionic mass under the different stressant regimes.

CHAPTERULE VII 5

SOMATIC WEIGHT:

A CRITIQUE

That the somatic weight of the crab, O. senex senex is not significantly altered under the different regimes of Cd and pH has been mentioned earlier (Chapter VI). In other words, under the stressant regimes, at the holontic (whole-organismal) level, there is an overall conservation of weight

status. Do the histogravimetric insights obtained in the present work shed any light on this aspect of 'somatic gravistasis' occurring in the crab under different stressant regimes ?

The total of HSIs of hepatopancreas, gill, chelate leg muscle and ovary in the 'control' (unstressed) crab equals 27.185 g (Table VII 5.29).

TABLE VII 5.29: Totals of wet HSIs of tissues of O. senex senex under the different stressant regimes.

(Data taken from Table VII 2.1)

Stress	Wet weight contributed by				Total (g)
	HP (g)	G (g)	M (g)	O (g)	
C	8.012	1.430	17.020	0.723	27.185
Cd 15 dps	3.650	1.343	18.970	3.340	27.303
Cd 30 dps	5.800	2.500	19.120	1.760	29.182
pH 15 dps	4.450	1.550	19.800	1.070	26.960
pH 30 dps	4.900	2.600	18.800	1.700	28.000
Comb 15 dps	5.007	1.560	18.600	1.200	26.367
Comb 30 dps	4.210	1.415	18.000	0.550	24.175

The 'totals' of the four tissue-weights under different stressant regimes do not vary much away from the 'control total' except under Cd 30 dps regime where a good elevation of the total is noted and under comb 30 dps regime where a good depression of the total is noted. These insights may suggest that somatic gravistasis involves a good degree of soft tissue weight conservation.

In the crab, the index of hepatopaneas is remarkably depressed. The increases of HSIs of chelate leg muscle and ovary under different stressant regimes, appear to compensate for the hepatopaneatic weight de-scalation mentioned. In certain cases an 'over compensation' is felt. For example, under Cd 30 dps regime, the chelate leg muscle and ovary show remarkable increases in their HSI status so that the total of the HSIs of the four tissues under this regime is greater than the corresponding value under control regime (C = 27.188; Cd 30 dps: 29.182; Table VII 5.29).

The totals of dry HSIs of the tissues (Table VII 5.30) also show similar weight conservation and compensation trends.

TABLE VII 5.30: Totals of dry HSIs of tissues of O. senex senex under the different stressant regimes.

(Data taken from Table VII 2.1)

Stress	Dry weight contributed by				Total (g)
	HP (g)	G (g)	M (g)	O (g)	
C	3.250	0.180	8.760	0.300	12.490
Cd 15 dps	1.423	0.190	9.212	1.421	12.246
Cd 30 dps	2.700	0.243	10.522	0.890	14.355
pH 15 dps	1.900	0.200	9.300	0.500	11.900
pH 30 dps	1.750	0.173	10.570	0.534	13.027
Comb 15 dps	1.806	0.231	7.700	0.620	10.357
Comb 30 dps	1.410	0.203	8.000	0.160	9.773

The totals of water-somatic indices (WSIs) of tissues (Table VII 5.31) illustrate 'conservation' of this complementary aspect of tissue wet weight. This trend of conservation of total of tissue weights (including the dry and hydrational complements) visualised with four tissues of the organism in the present work, may safely be presumed to be prevalent in the other tissues of the

TABLE VII 5.31: Totals of water-somatic indices (WSIs) of O. senex senex under the different stressant regimes.

(Data taken from Table VII 2.1)

Stress	Water contributed by				Total (g)
	HP (g)	G (g)	M (g)	O (g)	
C	4.760	1.250	8.250	0.425	14.697
Cd 15 dps	2.230	1.154	9.760	1.920	15.058
Cd 30 dps	3.100	2.243	8.600	0.880	14.835
pH 15 dps	2.730	1.360	10.500	0.600	15.090
pH 30 dps	3.160	2.400	8.300	1.150	14.957
Comb 15 dps	3.200	1.330	10.900	0.600	16.030
Comb 30 dps	2.500	1.211	10.030	0.400	14.412

organism, so that this total conservation picture accounts for and accords well with the somatic weight conservation noted above under different stressant regimes. Thus, somatic weight status of the organism under the so called stressant regimes appears 'healthy'.

But at the individual tissue level one may not fail to notice a deviation from 'health' in the form of reduction of the size of hepatopancreas (see chapter VI). This tissue being the 'central metabolic organ' in the organism, its weight-decrement should have deeper implication in the 'metabolic milieu' of the organism under toxicant duress.

The somatic weight conservation
or 'somatic gravistasis' noted in

VII 5.A CHAPTERULAR the crab, O. senex senex under
RE'SUME' the different regimes of Cd and
 pH, appears to have contribution
from the tissues that go into its organisation. Although
this may denote the 'health' of the organism under toxicant duress, individual tissue 'close-up' suggests the
situation to be one of only 'apparent health'.

INTERACTION OF
STRESSANTS

The work presented in
this dissertation is
VII 6.A PREFATORY aimed, amongst other
GROUND things, to make a con-
tribution towards elu-
cidation of the phenomenology of interac-
tion of stressants. But, in the earlier
locations, this aspect has not been
attempted to be elucidated. The reason
for this omission is that the data on the

weight-specific basis as presented in the earlier location of the thesis do not possess the additional 'elucidative' dimension viz., 'holohistometric approach'. Now that the data have been treated this way (see tables VII 4.20 to 26); the ground may now be deemed to be ready for the 'inspection' into the data to unearth the insights about interaction. Visualization of the phenomenology needs an appropriate recasting of the holohistometric data. This involves placing of the changes under Cd, pH and combinational regimes side by side, so that interaction examination can be conveniently made. Table VII 6.32 provides such datal disposition.

Classical interaction-descriptive terms like potentiation and antagonism appears to be applicable here only laconically. There are several shades of interaction in the data given in table VII 6.32 which are deviant from the definitions of potentiation and antagonism. Description of these shades require framing of appropriate phraseology.

Amongst the data, one may
find three modes:

VII 6.B PHRASEOLOGY AND PHRASEOGRAPHY

(1) Positive mode: This
includes data in which

variation under all the three regimes (i.e., Cd-, pH- and Comb- regimes) is on the positive or elevation side (POSI-MODE).

- 2) Negative mode: This includes data in which variation under the three regimes lies on the negative or depression side (NEGA-MODE).
- 3) Mixed mode: This includes data in which variation under the three regimes lies on both positive and negative sides of the control (centum-per-centum) line (MIXO-MODE).

Under the three modes visualised above, several shades of variation can be identified (Phraseology table-Table VII 6.33). The phraseological nuances, are graphically denoted in the phraseographic diagram (Fig VII 6.2)

TABLE VII 6.33: Phraseology table including positive mode (POSIMODE), negative mode (NEGAMODE) and mixed mode (MIXOMODE)

A. POSIMODE AND NEGAMODE

Trend	Phraseology numbers in	
	POSIMODE	NEGAMODE
a) Change under Comb regime less than the aggregate of changes under Cd and pH regimes	1	5
b) Change under Comb regime equal to the aggregate of change under Cd and pH regimes	2	6
c) Change under Comb regime exceeding the aggregate of changes under Cd and pH regimes	3	7
d) Change under Comb regime 2- times the aggregate of changes under Cd and pH regimes	4	8

(Contd.)

TABLE VII 6.33 (Contd)

B. MIXOMODE

Trends of changes under regimes			Phraseology number
Cd	pH	Comb	
a) Elevation	Elevation	Depression	9
b) Depression	Depression	Elevation	10
c) Elevation	Depression	Depression	11
d) Depression	Elevation	Elevation	12
e) Elevation	Depression	Elevation	13
f) Depression	Elevation	Depression	14
g) Elevation/ Depression	Elevation/ Depression	*'Zero' % Change	15

*Zero Change or a deviation of 5% about control line is taken as zero percent change for evaluation of trends



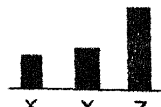
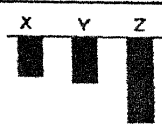

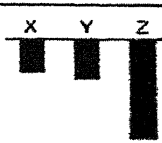

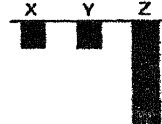
VII 6.C APPLICATION IN
THE PRESENT
SITUATION

The phraseology formulated above, may now be applied to the tissue situations in the crab O. senex senex under the stressant regimes. These







phraseologic (and phraseographic) readings into the data given in table VII 6.32 are appended in that table itself as the terminal column.

FIG. VII.6 2: INTERACTION PHRASEOGRAMS (IAPG) FOR USE IN CATEGORIZATION OF DATA OBTAINED FOR THE DIFFERENT ISSUES OF Q SENEX SENEX UNDER DIFFERENT STRESSANT REGIMES

(A) POSI- AND NEGAMODES

TREND NOTATION X = Cd, Y = pH, Z = Comb.	POSIMODE		NEGAMODE	
	IAPG category	DEPICTION	IAPG category	DEPICTION
$Z \leftarrow X + Y$	1		5	
$Z = X + Y$	2		6	
$Z > X + Y$	3		7	
$Z = n(X + Y)$	4		8	

(B) MIXOMODE

TREND NOTATION X = Cd, Y = pH, Z = Comb.	IAPG category	DEPICTION	TREND TRANSLATION (In stressant terms)
$X \uparrow \quad Y \uparrow \quad Z \downarrow$	9		CdE pH E Comb. D
$X \downarrow \quad Y \downarrow \quad Z \uparrow$	10		Cd D pH D Comb. E
$X \uparrow \quad Y \downarrow \quad Z \downarrow$	11		CdE pH D Comb D
$X \downarrow \quad Y \uparrow \quad Z \uparrow$	12		Cd D pH E Comb E
$X \uparrow \quad Y \downarrow \quad Z \uparrow$	13		CdE pH D Comb E
$X \downarrow \quad Y \uparrow \quad Z \downarrow$	14		Cd D pH E Comb D
$X \uparrow \downarrow \quad Y \uparrow \downarrow \quad Z = 0$	15	—	Cd _D ^E pH _D ^E Comb. '0'

A look-stretch at the interaction phraseographic (IAPG) readings into the data in table VII 6.32 may convince the critical reader about the diversity of interaction and the absence of any consistency of interpretatory legibility.

The 'posimode' and 'negamode' phraseographic data illustrate interaction which is 'ipsophasic' (alterations under all regimes belonging to the same phase) and the mixomode data illustrate 'contraphasic' (alterations under the regimes falling under different phases) interaction.

In the case of contraphasic interaction, the 'interaction' in combinational regime, can be interpreted, basing on the phases under Cd- and pH- regimes vis-a-vis the phase under combinational regime.

a) 'Phase-change' type of interaction: In this interaction, the changes under the individual (i.e., Cd and pH) regimes belong to one phase and in the combinational regime the change belongs to the opposite phase.

Phraseographic categories 9 and 10 (Fig VII 6.2) belong to this type of interaction.

b) 'Phase-mimesis' type of interaction: In this interaction, the phase of change under the combinational

regime mimicks the phase (of change) pertaining to one of the individual stressant regimes. This type of interaction is exemplified by the phraseographic categories 11 to 14.

c) 'Phase null' type of interaction: In this interaction, irrespective of the phases of changes under the individual regimes of stressants, the change under the combinational regime happens to be 'nil' or it falls into the 'null' range arbitrarily defined here (please see the asterisked foot note under table VII 6.33). This type of interaction is exemplified by the phraseographic category 15.

In the case of ipsiphasic interaction, often the interaction is succinc⁺. This is exemplified by the interaction phraseographic (IAPG) categories 1, 2, 3, 5 6 and 7 (Fig VII 6.2).

In the IAPG-categories 1 and 5, there is a bit of 'damping' of the effects of the stressants in the combinational regime. If X represents the quantum of effect under Cd regime, and Y, the quantum of effect under pH regime, then Z the quantum of effect under the combinational regime will be less than the cumulated quantum of effects of individual regimes (1)

$$Z < X + Y \quad \dots \quad (1)$$

In the IAPG-categories 2 and 6, both stressants or the effects are 'equally asserted' in the combinational regime. In other words, the quantum of effect under the combinational regime equals the cumulation of the quanta of effects under the individual regimes (ii)

$$Z = X + Y \quad .. \quad .. \quad (ii)$$

In the IAPG-categories 3 and 7, the quantum of effect under the combinational regime exceeds the cumulation of quanta of individual-regime-effects, but not 'excessively' (see below) (iii)

$$Z > X + Y \quad .. \quad .. \quad (iii)$$

Finally, in the IAPG-categories 4 and 8, the quantum of effect under the combinational regime exceeds, 'excessively' the cumulative quantum of individual-regime-effects. More specifically, the 'combinational effect' appears as a duple or a multiple of the cumulative quantum of individual-regime-effects (iv)

$$Z = n (X + Y) \quad .. \quad .. \quad (iv)$$

The account given above may appear more as some odd theoretical reading, less germane to the subtitle of this section of the chapterule. But the account has to be that way: The absence of consistency of interaction trends has been mentioned above. Such an inconsis-

tency, naturally prevents one from developing a concise and cogent interpretatory theme. In such a circumstance, the approach of phraseological and phraseographical 'theoreogenesis' has been adopted as an anaplerotic alternative. With this phraseographic categorization background, one may stop at each of the horde of interaction locations recorded in table VII 6.32 and have an individual interpretatory look at it.

However much the IAPG-record circumstances in table VII 6.32 may look inconsistent, the critical eye may not fail to notice some bright 'spots' consistency amongst these data.

1) Hepatopancreatic TCHR (total carbohydrate reserves) data and TAEAPS (total acid-extractable anthrone-positive substances) belong to the phraseographic category 5, under both temporal regimes (15 dps and 30 dps). The negative (negamode) ipsophasic interaction in these cases is the 'damping' influence of the combinational presence of the stressants Cd and pH, as compared to the individual regimes of the stressants.

2) The hepatopancreatic dehydrogenases show the general pattern of incidence of IAPG-category 11 in the shorter stress-duration regime and IAPG-category 13 in the longer stress-duration regime. Thus, in the

HP-dehydrogenase situation, the depressive picture of combinational regime in shorter stress-duration is replaced by elevatory picture in the longer stress-duration. In other words, the combinational regimes of Cd and pH depress the dehydrogenase system during shorter-term exposures and elevate this system during longer-term exposures.

3) The total ninhydrin-positive substances (TNPS) of the branchial tissue (gill) of the crab belong to the IAPG-category 1. In this tissue, this constituent undergoes elevation in both stressant regimes, during both temporal durations. In the combinational regime, the elevatory effect is 'damped' to a considerable extent. In other words, the quantum of combinational 'elevatory' effect in both temporal durations on the TNPS pool of gill is considerably smaller than the cumulation of the quanta of elevatory effects under the Cd and pH regimes in severo.

4) The branchial total acid-precipitable anthrone-positive substances (TAPAPS) belong to the IAPG-category 9 in their interaction profiles. This category stands for elevation of the constituent under both in severo Cd- and pH- regimes. In the combinational regime, this elevatory effect is replaced by depressory effect.

In other words, the combinational regime of the stressants functions as a 'mobilizer' or 'metabolizer' of the TAPAPS-component of TCHR pool of this tissue.

5) The adenosine triphosphatase (ATPase) system of branchial tissue shows the general pattern of belonging to IAPG-category 11 in the interaction profile.

This category includes changes of negative direction under Cd-regime and changes of positive direction under pH-regime. In the combinational regime a pH-effect mimesis is noted. Thus, under the combinational regime, the negative effect of Cd-regime is suppressed and the positive effect of pH-regime is expressed.

6) The succinate dehydrogenase activity of branchial tissue belongs to IAPG-category 14 in its interaction profile. In this case, the Cd-regime causes negative effect on the activity of SDH and pH-regime, a positive effect. In the combinational regime, a Cd-effect mimesis is evident. In other words, in the combinational regime, the positive effect of pH-regime is suppressed and the negative effect of Cd-regime is expressed.

7) In the chelate leg muscle tissue, the soluble protein component of total protein pool belongs to the IAPG-category 1 in its interaction profile. This is an

interaction involving 'damping' of individual-regime-effects in the combinational regime. Thus, in this tissue, this biochemical component is influenced by the stressants Cd and pH in the combinational regime, where they 'shed' to a considerable extent their individual tissue-ISP-augmentative influences, so that the net effect under the combinational regime is considerably less than the cumulation of the effects of Cd and pH in severo.

8) The total lipid pool (TL) of chelate leg muscle belongs to IAPG-category 12 in the interaction profile under both shorter and longer stress-durations. In this case, the Cd-effect is negative. The pH-effect is positive. In the combinational regime, positive effect is noted (pH-effect-mimesis). The interpretatory ground is obvious.

9) The total carbohydrate reserves (TCHR) of chelate leg muscle belong to IAPG-category 7 in the interaction profile. In this negamode profile of IAPG, the negative effect of combinational regime is greater than the cumulation of the negative effects of the individual stressant regimes.

10) The activity of alanine aminotransferase (ALAT) of chelate leg muscle belongs to IAPG-category 1

in the shorter stress-duration regime and IAPG-category 3 in the longer stress-duration regime.

The posimode 'damping' type of interaction in the shorter stress-duration stands for one type of interaction and the posimode 'augmentative' type of interaction in the longer stress-duration stands for a different type of interaction.

11) The activity levels of total ATPase and Mg^{2+} -ATPase of chelate leg muscle show IAPG-category pattern including incidence of category 1 in the shorter stress-duration and category 3 in the longer stress-duration. These parameters, thus, show 'damping' type of interaction profile in the shorter stress-duration and 'excessory' profile in the longer stress-duration.

12) Succinate dehydrogenase activity of chelate leg muscle belongs to IAPG-category 10 in the stressant interaction profile. The individual regimes are associated with notable depressions of the levels of enzyme activity. In the combinational regime, there is switchover to posimode phase of enzyme activity modification, that too, to a remarkable extent. This phase in combinational regime of chelate leg muscle may be contrasted with 'negamode' modulation met with in this regime for this enzyme in hepatopancreas and branchial tissue. This contrast marks

the leg muscle out from the other tissues — a 'touch' of tissue personality or tissue specificity.

13) The acetylcholine esterase (AChE) activity of chelate leg muscle belongs to IAPG-category 1 in both longer and shorter stress-durations. There is a considerable quantum of 'damping' of posimode effects of the stressants Cd and pH in combinational regime.

Besides these IAPG-category readings, which involve only 'mild' 'interactions' in the combinational regime, there are two instances in which the interactions are not mild: In the combinational regimes, in these instances, the quantum of effect is a multiple of the cumulative quanta of effects under in severo regimes. The instances are (1) 15 dps soluble protein content in the branchial tissue and (2) 30 dps LDH-activity of the same tissue. In the case of the former parameter, the interaction belongs to IAPG-category 4 and in the case of the latter, the IAPG-category is 8.

These instances may be taken as potentiatory interactions qualifying for application of the term 'synergism'.

What general category the multitude of interactions or the IAPG-categories elaborated above belong to, is left to the 'phrontistery' of the critical reader-fraternity.

The action of Cd on the various facets of organismic biology and biochemistry is reported by several workers. Some works pertain to human and mammalian histopathology and physiological toxicology (Severi, 1896; Prodan, 1932; Wilson et al., 1941; Schroeder et al., 1965; Lewis et al., 1969; Gilluly, 1970; Nilsson, 1970; Stowe et al., 1972; Itokawa, 1973; Wald et al., 1974; Nomiya, 1975; Nechay and Saunders, 1977; 1978; Nogawa et al., 1979; Taniguchi et al., 1979) while others pertain to submammalian toxicology (Gardner and Yevich, 1970; Sangalang and Freeman, 1974; Christensen, 1975; Larsson, 1975; Koyama and Itazawa, 1977; Piavaux, 1977; Johansson and Larsson, 1978; Tucker, 1979). Other works provide insights into the detoxification mechanisms available for offsetting the toxic influence of metals like Cd by a 'stow-away' mechanism of 'metal binding' (Siebers and Ehlers, 1978; Briggs, 1979; Pecon and Powell, 1981).

Similarly, the 'numerical' toxicology of pH (Parsons, 1968; Sutchliffe and Carrick, 1973; Almer et al., 1974; Grahm et al., 1974; Hendrey and Wright, 1975, 1976; Karuppasamy, 1979; Hoback and Raddum, 1980; Parent and Cheetham, 1980; Okland, 1980; Raddum, 1980; Miller and Mackay, 1980; Murthy et al., 1981a, b; Walton et al., 1982; Mastanamma, 1984) and the physiological and biochemical toxicology of pH (Calabrese,

1969; Savita Samant and Agarwal, 1977; Mastanamma, 1984) have been worked out in some detail.

But these research readings put together may not be of any particular help in interpretation of interaction phenomenology in the present organism.

In the organism no interaction phenomenon has been found to be of reasonable consistency tissue-wise, enzyme-wise or organic-constituent-wise. In such a 'loose-ground' the points of profiles of interaction can be identified with some difficulty.

The phenomenon of potentiation which denotes an intense interaction between the stressants has been noted in too few instances to be of any interaction-clarification help.

The antagonistic interaction is, on the other hand, instanced as numerous hazy-profiled pictures. Except the IAPG-categories 7 and 8, all the other IAPG-categories recorded in the present work can be included under the broad umbra of antagonism.

One reassuring point from the organismic survival angle is essentially the absence of any serious interaction between the stressants considered above. But then this reassurance can only be of a small avail as the reader will note in a succeeding chapterule.

1. Interaction between the stressants Cd and pH in their combinational regime is visualized in the different organic components and enzyme-activity levels in the tissues of the crab, O. senex senex.

2. A special approach of phraseology and phraseography has been evolved here to identify the interaction phenomena in the tissues of the organism.

3. Instances of classical synergistic interaction are available in only two locations (viz., in the case of soluble protein content, 15 dps and LDH-activity, 30 dps, of the branchial tissue).

4. Other interaction phraseographic categories (IAPG-categories) recorded in the present work can be included under the broad umbra of antagonistic interaction.

CHAPTERULE VII 7

SOME CORRELATIONS

The preceding chapterule has given the
VII 7.A PREFATORY picture of the absence
of consistent interaction profiles between the stressants in
the tissues of O. senex senex. But, it
can be said in a general way that the
three regimes (including Cd, pH and the
so-called interaction regime, the combi-
national regime) share between them

modulatory (enzyme activity) and modificatory (organic component level) influences of comparable magnitude. This is one aspect.

The second aspect is contained in the following interrogative: Does there exist any 'interaction', cause-and-effect relation or some such phenomenology between the different components, constituent and catalytic machinery of the tissues underlining modulations and modifications caused by the stressant regimes ?

	The above-noted interroga-
VII 7.B THE CORRELATIONS	tive forms the action-cause
i) TCHR AND SDH	for examination of the phe-
	nomenon of covariation bet-
	ween different components and machineries at 'sub-
	histontic' (at the intra-tissue) level and its statis-
	tical quantification.

Tables VII 7.34, 35 and 36 provide results of such 'statisticoscopic' examination.

In hepatopancreas (Table VII 7.34), branchial tissue (Table VII 7.35) and the chelate leg muscle (Table VII 7.36), a negative covariation has been identified between the activity of succinate dehydrogenase, the indicator enzyme for the Krebs cycle

TABLE VII 7.34: Study of covariation between TCHR and SDH in hepatopancreas of O. senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from tables VII 5.20 and VII 5.23).

Regime		TCHR	SDH
Cd	15 dps	123.0	1218
pH	15 dps	118.9	324
Comb	15 dps	111.2	288
Cd	30 dps	166.4	834
pH	30 dps	132.8	523
Comb	30 dps	160.0	303
C		313.0	397
$r = -0.355; NS$			

activity and the total carbohydrate reserves (TCHR) of the tissues. The correlation coefficient obtained from the statistical covariation analysis for the tissues are in the following decreasing order: M($r = -0.43$) HP($r = -0.355$) G($r = -0.115$). In all the three cases, however, the 'r' values have not been found to be statistically significant.

TABLE VII 7.35: Study of covariation between TCHR and SDH in gill of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from tables VII 5.21 and VII 5.24).

Regime	TCHR	SDH
Cd 15 dps	33.1	118
pH 15 dps	54.3	234
Comb 15 dps	32.0	169
Cd 30 dps	76.3	105
pH 30 dps	61.1	372
Comb 30 dps	23.3	318
C	58.5	215

$$r = -0.115$$

NS

What is the meaning of this negative co-variation between SDH and TCHR especially in the cases of HP and M in which the 'r' values are more pronounced than in the case of G ? That the depression of SDH is associated with the conservation of TCHR is the plain inference that

TABLE VII 7.36: Study of covariation between TCHR and SDH in muscle of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from tables VII 5.22 and VII 5.25).

Regime		TCHR	SDH
Cd	15 dps	501	348
pH	15 dps	752	744
Comb	15 dps	236	1050
Cd	30 dps	726	508
pH	30 dps	401	214
Comb	30 dps	281	2176
C		793	823
- - - 0.43		NS	

one can draw from this covariation. This inference may be juxtaposed with the observation made elsewhere that the total acid-extractable anthrone-positive substances (TAEAPS) fraction of the TCHR pool of tissues is 'burnt' out excessively under the different stressant regimes (please vide chapter IV). Does it not reflect

a 'compensation' by one fraction of the TCHR for the 'loss' suffered by one of the fractions? Such inherent compensatory mechanisms undoubtedly aid the organism/suborganismal components in playing down the toxic influence of stressing elements.

The cause-and-effect relation between the aminotranferases VII 7.4(ii) TAT AND TNPS and the total ninhydrin positive substances (TNPS) has been mentioned in an earlier context (see Chapter V). The aminotransferase relative activity (ATRA = The ratio between AAT activity and ALAT activity) or the De Ritis quotient undergoes significant alteration under the different stressant regimes, suggesting influence of the stressants on the 'metabolism intergression points' (see Chapter VII 3). Since both the aminotransferases are concerned with the metabolism of aminoacids, an examination of relation, if any, between the total aminotransferases activity (TAT) and TNPS pool which includes the free amino acids of the tissue will be in order (Tables VII 7.37 and VII 7.38).

In hepatopancreas, a negative covariation picture is obtained ($r = -0.48$; Table VII 7.37) whereas in the case of chelate leg muscle a positive covariation

picture emerges ($r = +0.074$; Table VII 7.38). The data, prima facie, may reflect on a basic 'personality' difference between the two tissues with regard to the mode of

TABLE VII 7.37: Study of covariation between TAT and TNPS in hepatopancreas of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from tables VII 5.20 and VII 5.23).

Regime		TAT	TNPS
Cd	15 dps	5174	1048
pH	15 dps	1373	3087
Comb	15 dps	2629	1051
Cd	30 dps	2118	1456
pH	30 dps	7111	1490
Comb	30 dps	5252	1587
C		2059	1883

= - 0.48 NS

modification of TNPS pool vis-a-vis total aminotransferase activity. In hepatopancreas, the negative covariation between TAT and TNPS speaks for the 'negative'

TABLE VII 7.38: Study of covariation between TAT and TNPS in chelate leg muscle of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from tables VII 5.22 and VII 5.25).

Regime	TAT	TNPS
Cd 15 dps	6521	8062
pH 15 dps	4406	6534
Comb 15 dps	7914	3050
Cd 30 dps	4133	5736
pH 30 dps	13056	8009
Comb 30 dps	11584	10170
C	1981	5446

$$r = + 0.074 \quad \text{NS}$$

role played by the enzyme-complement in mention on the level of the organic component in question in the tissue under interpretation-focus.

In the case of chelate leg muscle, the TAT complement appears to play a less negative role in the area of TNPS-level modification, if not, a vehemently augmentative role.

In the absence of further and more incisive experimental evidence, the cause and effect correlation between TAT and TNPS in the tissues in question may not be examined in fuller perspective.

The enzymes, succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) are the primary catalytic signposts notifying intra-organismal and intra-histontic (inside the individual tissue) energetic state. Examination of these signposts under stressant regimes has proven interpretationally useful (see Chapterules VII 5 and VII 6). Examination of these 'signposts' and their ele-dep (levation-depression) nuances under the different stressant regimes, in 'tabular' proximity (Tables VII 7.39 to VII 7.41) may prove more useful.

In the case of hepatopancreas (Table VII 7.39) and branchial tissue (Table VII 7.40), the covariation between these 'enzymic signposts' is found to be negative (hepatopancreas: $r = -0.142$; branchial tissue:

$r = -0.208$). In the chelate leg muscle, on the contrary, a positive covariation is found ($r = +0.63$; Table VII 7.41).

TABLE VII 7.39: Study of covariation between hepato-pancreatic SDH and T.ATPase in O. senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from Table VII 5.23).

Regime	SDH	T.ATPase
Cd 15 dps	1218	2499
pH 15 dps	324	1652
Comb 15 dps	288	1926
Cd 30 dps	834	1303
pH 30 dps	523	980
Comb 30 dps	303	4050
C	397	2336

$r = -0.142$

NS

This contrast in covariation profile between chelate leg muscle on the one side and hepatopancreas and branchial tissue on the other side serves to provide

TABLE VII 7.40: Study of covariation between branchial SDH and T.ATPase in O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from table VII 5.24).

Regime		SDH	T.ATPase
Cd	15 dps	118	127
pH	15 dps	234	263
Coru	15 dps	169	309
Cd	30 dps	105	200
pH	30 dps	372	224
Corb	30 dps	318	350
C		215	202

r = -0.208		NS	

one more highlight to the physiological and biochemical specificities which have been touched at several earlier locations in this dissertation.

In hepatopancreas and branchial tissue, the relation between SDH and ATPase system, statistically characterized by negative correlation, may be described

TABLE VII 7.41: Study of covariation between chelate
leg muscular SDH and T.ATPase in O.
senex senex with reference to the
different regimes of Cd and pH.

--- (Date for analysis taken from table VII 5.25). ---

Regime	SDH	T.ATPase
Cd 15 dps	348	8804
pH 15 dps	744	11166
Comb 15 dps	1050	5987
Cd 30 dps	508	6665
pH 30 dps	214	7276
Comb 30 dps	2176	15444
	823	3220

= + 0.63 NS

as fritter-relation. ATPase being ATP-level-decreasing enzyme, its elevation leads to depletion of tissue-ATP reserves. SDH — the Krebs cycle activity indicator enzyme, through its depression under condition of elevation of ATPase system, renders the energetic situation more serious, since depression of this enzyme means an overall depression of ATP-genesis. Such a situation

by incidence in the two tissues which are at two important 'metabolic gateways' — renders the whole-organismal metabolic status under the stressant regimes, more shaky.

The situation of chelate leg muscular energetics with positive covariation between SDH and T.ATPase should interpretably be healthier under the toxic regimes of stressants. This 'trait' of muscle may render it less pervious to the energetic depression overtaking the other tissues of the organism under the stressant-duress.

The special physiological personality profile of excitability of muscular and neural tissues is

VII 7.3(iv) AChE AND
NON-Mg²⁺-ATPase

known to be underlied by

the characteristic esterase, acetylcholine esterase (AChE)

The other enzyme, playing equally important role in sustaining the excitability of these two tissues is Na⁺-K⁺

ATPase. Do the data of the present work on crab's tissues throw any light on the relationship between this duo of excitability-state-maintenance-enzyme-agents (ESMEA) ?

In both chelate leg muscle (Table VII 7.42) and the cephalothoracic ganglionic mass (Table VII 7.43), one gets fairly convincing evidence for a 'working' relation between 'ESMEA' duo. In the case of the cehalo-

TABLE VII 7.42: Study of covariation between AChE and non-Mg²⁺-ATPase of chelate leg muscle of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from table VII 5.25).

Regime		AChE	Non-Mg ²⁺ -ATPase
Cd	15 dps	235425	6321
pH	15 dps	234486	6327
Comb	15 dps	128005	1857
Cd	30 dps	323748	5015
pH	30 dps	105496	2830
Comb	30 dps	261846	10074
C		69840	2182
r = + 0.73		NS	

thoracic ganglionic mass, the evidence is more appealing with a significant (5%) correlation co-efficient ($r = +0.96$) obtained through correlation analysis. That a 'relation' between 'ESMEA duo' should be gleaned through the data obtained on several diverse stressant

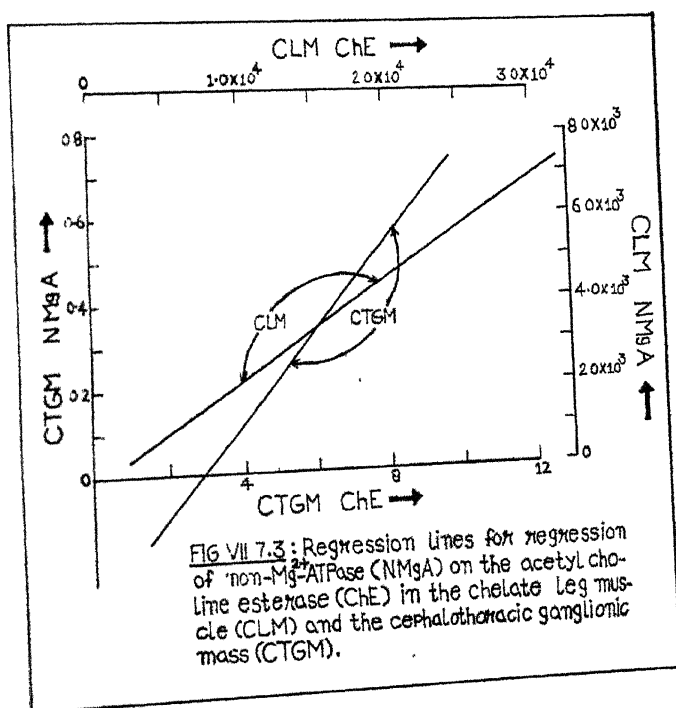
TABLE VII 7.43: Study of covariation between AChE and and non-Mg²⁺-ATPase activity of cephalothoracic ganglionic mass of O. senex senex with reference to the different regimes of Cd and pH.

(Data for analysis taken from table VII 2.1).

Regime		AChE	Non-Mg ²⁺ -ATPase
Cd	15 dps	3.60	0.021
pH	15 dps	2.34	0.020
Comb	15 dps	3.40	0.003
Cd	30 dps	3.81	0.061
pH	30 dps	4.30	0.059
Comb	30 dps	9.00	0.681
C		3.25	0.121
r = + 0.96		S 5%	

regimes should argue for the perseity and perseverance of this relation, through the thick and thin of variable stressant impact on the various facets of histontic (individual) metabolism. This interesting covariation

may be given another statistical 'conspection' (Table VII 7.44) to obtain a graphical picture of the relation between non-Mg²⁺-ATPase and AChE (Fig. VII 7.3).



i) Analysis of correlation

between succinate dehydrogenase (SDH) and total carbohydrate reserves (TCHR)

VII 7.C CHAPTERULAR RE'SUME'

in hepatopancreas (HP), chelate leg muscle (M) and gill (G) of O. senex senex with reference to different regimes of Cd and pH reveals a trend of conservation of

TABLE VII 7.44: Regression analysis on the data given in tables VII 7.42 and VII 7.43

Issue	Regression Equation*	t**	p***
Cholito leg muscle	NMgA = 0.482+0.023 ChE	2.50	NS
Deglutatharacic pancreatic mass	NMgA = -0.315+0.107 ChE	7.64	0.05

*For describing regression of Non-Mg²⁺-ATPase (NMgA) on acetylcholine esterase (ChE)

**t : Calculated students' 't' test value for testing the significance of regression co-efficient.

***p : Level of significance; NS : Not Significant

reserves vis-a-vis SDH, indicated by negative covariation between these 'paired'-up parameters. The correlation coefficient (r) is in the decreasing order in the tissues as follows: M < HP < G.

ii) Covariation relation between total of aminotransferases (TAT) and total ninhydrin-positive substances (TNPS) is negative in HP and positive in M. This difference is suggestive of 'personality' differences between HP and M in the area of nitrogenous

iii) In the cephalothoracic ganglionic mass (CTGM) and chelate leg muscle (CLM), the covariation between acetylcholine esterase (AChE) and non-Mg²⁺-ATPase, the 'excitability-state-maintenance-enzyme-agent (ESMEA) duo' is found to be positive and in the case of CTGM the relation is found to be statistically significant.

CHAPTERULE VII 8

NUTRIENT

METAQUANTIGRAPHY

(NIQG)

In this chapter, in
the various chapte-
VII 8.A EXPLORATORY rules thus far, the
facets of tissue
nutrients and enzyme activities have been
examined in only 2 dimensions: stress-
duration dimension and stressant-nature
dimension. Thus far the consideration
happens to have an 'area' but devoid of
the third dimension, the 'depth'. A con-

sideration of 'inter-tissue transactions' is the other depth dimension. An attempt will be made to 'measure' this 'depth' in this chapter.

VII 8.B THE TERM In the histontic metabolism, metabolites (nutrients) and the catalytic enzymic agents are involved closely and often the 'metabolite levels' are employed as indicators of the metabolic profile of the tissue. Alteration of the 'metabolite levels' occur through a maze of elevation-depression profiles the enzymes undergo. Which is why the 'metabolite level' may be regarded as the quantitative 'expression' of enzyme catalytic function.

The 'metabolite level' is also underlined by another causo-mechanism — inter-tissue transaction.

Thus, a nutrient pool (its size) is influenced by two contributory factors.

- i) tissue's own metabolism (endogenous metabolism) and its direction (synthesis/degradation)
- ii) the tissue's transactions with the other tissues in the intra-organismal milieu.

Changes in the quantities of tissue nutrient pools under given experimental situation and their

pictorial disposition will facilitate examination of the phenomenology of inter-tissue transaction. This change in quantity picturisation approach is proposed to be called tissue nutrient meta-quantigraphy (NMQC). The results of such an exercise are provided in fig. VII 8.4 as 'metaquantigrams' for the different stressant regimes. Witness the varied metaquantigraphic profiles of tissues under the different stressant regimes. The reader may examine these profiles keeping in mind the well reported 'metabolic catholicity' of hepatopancreas as against the metabolic conservatism tissue like muscle.

The uniform theme of all the NMQGs is the elevation of lipid pool of haemolymph irrespective of the direction of change of this constituent in other tissues. What is the significance of this 'hyperlipnoemic' effect of the stressant regimes ?

The lipid pool of hepatopancreas is generally depressed considerably, under all the regimes. What contribution does this decrease make to the metabolic pools of the other tissues ?

The TCNR pool of tissues is generally depressed in all the tissues. Here, as also in the case of lipid pools, the inter-tissue transactions are not 'visionable'.

Fig VII 8.4:

Nutrient meta-quantigrams (NMQG) of the tissues of O. senex senex under different regimes of Cd and pH.

To perceive the NMQG of any regime, the reader may reach the appropriate page of the NMQG 'booklet' and 'fold' the 'transparent cardinal silhouette' over the NMQG and lo! the changes in the nutrient quantities in the tissues will be 'apparent' in the 'cardinal confines' (Changes in quanta of nutrients depicted in NMQGs are in milligrams).

REGIME: Cd 15 dps

<u>HP</u>	<u>HL</u>	<u>M</u>	<u>G</u>
TP -238	-88	-1214	+63
TCHR -190	-10.2	-292	-25.4
TL -328	+113	-96	-29.4

REGIME : Cd 30 dps

<u>HP</u>	<u>HL</u>	<u>M</u>	<u>G</u>
TP +143	+150	+161	+ 252
TCHR -146.6	-3.3	-67	+17.8
TL -201	+200	-168	+55.5

REGIME: pH 15 dps

<u>HP</u>	<u>HL</u>	<u>M</u>	<u>G</u>
TP -586	-69	+1963	+14
TCHR -194	-7.2	-41	-4.2
TL -80	+100	+1044	+55.1

REGIME: pH 30 Δ 35

<u>HP</u>	<u>HL</u>	<u>H</u>	<u>G</u>
TP +101	+45	-787	+455
TCHR -180	-3.8	-392	+2.6
TL -196	+229	+566	-0.2

REGIME: Comb 15 dps

	<u>HP</u>	<u>HL</u>		<u>M</u>	<u>G</u>
TP	-394	-33		-2235	+111
TCHR	-201	+1.4		-55.7	-26.5
TL	-116	+196		+661	+55.5

REGIME: Comb 30 dps

<u>HP</u>	<u>HL</u>	<u>F</u>	<u>G</u>
TP -445	-143	-1637	+191
TCHR -153	-12	-512	-35.2
TL -404	+309	+90	+41.5

In the case of protein pools, there are situations where inter-tissue mobilisation may be circumstantially visualised. Under pH 15 dps regime, the 'loss' of TP of hepatopancreas may be traced to some extent, in the gain recorded in muscle. But there is more to this mere loss-gain relationship. In the same regime, the gain of TP in muscle (+ 1963 mg) is far in excess of 'losses' of TP in hepatopancreas (-586 mg) and haemolymph (-69 mg) put together. What is the meaning of this 'overbalance'?

Witness also, in the same regime, the great gain in TL pool of muscle which has the 'overbalance' tough to it.

These and other quanti-genic perceptions that may arise in the mind of the reader may render this exercise metaquantigraphy a useful exercise if not a wholesome one with regard to elucidation of the phenomenon of inter-tissue transaction. Other technical and experimental complements are warranted to throw more informative light of this phenomenology.

VII 8.C CHAPTERULAR
 RE'SUME'

i) The changes in quantities
 of nutrients in tissues
 are employed to construct
 nutrient metaquantigrams
 (NMQGs) of tissues of O. senex senex under different
 regimes of Cd and pH.

ii) The NMQGs are to some extent useful in
 visualizing the phenomenology of inter-tissue metabo-
 lite transactions.

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EPILOGUE

The reader has come
thus far through the
VII 9.A PRO-EPILOGUE preceding chapteral
and chapterular maze
of data. The author, now, is not sure
whether he has been able to establish a
wavelength of intelligibility with the
reader to communicate the datal import.
Amidst the amplitude of 'verbogenesis' or
technical term-genesis, some insights may

not escape notice. Precisely, for the purpose of identification of such insights, the author had to resort to the generation of terminology out of interpretational necessity. And, the apparent excess of this exercise arises out of the author's elucidational zeal in excelsis.

VII 9.E STRESS-INDUCED HYPOXIA COMMENTED	Hypoxia of tissues, appears to be a concomitance for 'anti-organisamal' agents in general, the stressants of the present work proving to be no exceptions. Reduction of the rate of oxygen consumption under the different stressant regimes noted in <u>O. senex senex</u> (see Chapter III), might have led to hypoxic condition near the 'tissue-theatre'. The concept of hypoxia is only an assumption which is visualizable through the indirect evidence of assessment of Krebs cycle activity. The present work includes data, suggesting reduced Krebs cycle activity, <u>if</u> the reductions in the activity levels of succinate dehydrogenase under the different stressant regimes in the tissues of the crab are any indication (see Table VII 2.1).
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The stressants, irrespective of their chemical taxonomic status, appear to share this oxygen-consumption depressive effect in common. In the same crab,

depression of whole-animal respiration (WAR) has been noted under hyperosmotic salinity stress (Venkata Reddy, 1976) and under pesticide stress (Bhagyalakshmi, 1981).

There are reports about the allosteric effect of lactate on the oxygen binding sites in haemolymph (Grahm et al., 1974; Truchot, 1980). This may explain the reduction of oxygen consumption, but, again the causal source of 'lactate' becomes an intractable problem. For, lactate accumulation presupposes hypoxia at the tissue level and reduction of oxygen consumption at the whole organismal level. Probably the stressant 'signal' is perceived by the animal's integrative system which brings into operation a similar 'metabolic reflex' in the organism irrespective of the chemical identity of the stressant. Once the 'pace' of reduction of oxygen consumption is set in this reflex way, the 'lactatogenous' deaffination in haemolymph for oxygen may act further to 'stabilize' this depression-of-metabolism trend. The trend of metabolism turn-around or stabilization at a lower level of activity around the third week of stress in the crab (see Chapter III) may be a reflection of the lactatogenous deaffination phase of the stressant influence on the organism.

VII 9.C Cd and pH: The data, presented in the earlier chapterular locations of this chapter and in the earlier chapteral locations, TOXICITY IN may reveal one point with COMPARATIVE regard to the nature of action of these toxicants: PERSPECTIVE that the stressants, Cd and pH, cause similar effects in the different tissue locations, on several occasions. The 'toxic-catalytic' profiles of the two stressants, therefore, seem to be similar. In this connection, one may note that both the stressants used in the present work belong to the cationic category. It is tempting to assume the 'cationic function' being involved in the causation of biochemical and catalytic tissue profiles noted here under the hegemony of the two stressants. The details of the physiological and biochemical toxification of the tissues is beyond the interpretational potential of the present data.

VII 9.D DETOXIFICATION: In several animal locations of metal intoxication, the THE ROLE OF small molecular-weight pro- HAEMOLYMPH LIPID teinaceous compounds, the 'metallothioneins' have been found to be persuasively involved in the 'detoxification efforts' of the biological system (Olafson and Thompson, 1974; Noel-Lambert, 1976; Jacobson and Turner,

1980; Durnam and Palmitter, 1981; Hallenbeck, 1984). The detoxification potential of metal chelants like aminoacids has also been appreciated in several cases (Siebers and Ehlers, 1978; Briggs, 1979; Pecon and Powell, 1981).

Visualization of such mechanisms, as are enumerated above, in the present organism, is beyond the interpretational import of the present data. But, one consistent hemochemical characteristic noted under the hegemony of both the stressants viz., 'hyperlipaemia', is too conspicuous to be ignored. A perusal of the nutrient meta-quantigram (NQGs) for the different regimes (Fig. VII 8.4) suggests that this hyperlipaemic propensity is independent of the directions of change of lipid content taking place in the other tissues. In the Cd 15 dps regime, the hyperlipaemic state is flanked by 'hypolipohistia' in hepatopaneas on one side and chelate leg muscle and branchial tissue on the other side. In the case of Cd 30 dps regime, hypolipohistia of hepatopaneas and chelate leg muscle show up against hyperlipaemia, while branchial lipid content shows elevation. In the pH 15 dps regime, hyperlipaemia is flanked by hypolipohistia of hepatopaneas and massive hyperlipohistia of chelate leg muscle. In the other

regimes also, the hyperlipaemic status shows up against similar hyperlipohistiatic states in chelate leg muscle and branchial tissue.

This consistent hyperlipaemia is strongly suggestive of some function for lipid in haemolymph in the stressant-intoxication context.

One is tempted to suggest tentatively the function of detoxification to this haemolymphatic constituent. The function of such a toxino-lipid, if any, in the haemolymphatic location, appears to have logistic soundness too. Toxino-lipid complexes can better be stored in the metabolically neutral tissues like haemolymph rather than in the solid tissues, where the complex may face the hazard of lysis and may cause hazard to the metabolic tissue machine. In other organisms, in other metabolic contingencies, similar 'toxin-stow-away' function is visualized for haemolymph. In the apple snail, Pila globosa during long term aestival stress ('claustrobiosis'), the haemolymph appears to be the safe storage site for various aestival metabolites (Chandrasekharam, 1986; unpublished data).

VII 9.E INTERMEDIATE
METABOLISM
UNDER STRESSANT-
DIRECTION

The data on biochemical com-
position and catalytic picture,
given in the earlier locations
of this dissertation, may not
fail to suggest to the discern-

ing reader, a turn or twist in the intermediate metabo-
lism. The considerable decrease of carbohydrate reserves
of the tissues and the depression of Krebs cycle activity
are favourable data ground for invocation of glycolysis
as the metabolic alternative under the stressant duress.
But then, the accumulation of protein and lipid in some
tissues under certain regimes (please peruse the NMOGs
given in fig. VII 8.4) warrant modification of this invo-
cation in the direction of hexose monophosphate (HMP)
shunt pathway, so that this may serve as supply source of
reducing equivalents, necessary for synthesis of lipid
and protein (Epilogogram I: Fig. VII 9.5).

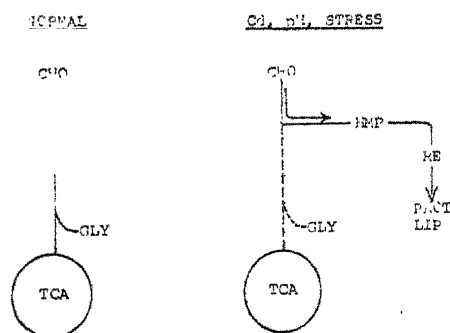


FIG. VII 9.5: EPICOLOGRAM I: IN THE NORMAL UNSTRESSED ORGANISM, THE CARBOHYDRATE (CHO) METABOLISM OCCURS THROUGH THE NORMALLY OPERATIONAL KREBS TRICARBOXYLIC ACID (TCA) CYCLE. UNDER STRESS (Cd, pH), THE METABOLISM IS DIVERTED AWAY FROM TCA CYCLE ABOVE GLYCOLYSIS (GLY) FROM CHO POINT INTO THE HEXOSEMONOPHOSPHATE SHUNT (HMP). THE REDUCING EQUIVALENTS (RE) RESULTING FROM HMP-SHUNT, CAN BE USED FOR THE SYNTHESIS OF PROTEIN (PROT) AND LIPID (LIP).

VII 9.F H-HH TRANSAC-
TIONS

The NMQGs, presented in Fig.
VII 8.4, show accumulation
of organic constituents under
certain regimes in certain
tissues. Such accumulations

may have major metabolic involvement (the situation
picturised in section VII 9.E may be recalled here).
Besides, inter-tissue exchanges also may have a role
to play in these accumulations, especially in metabo-
lically conservative tissues like muscle.

In various organisms including crustacean
arthropods, the hepatopancreas (= digestive gland) acts
as the central metabolic organ, exhibiting extensive
metabolic catholicity. It is involved in the supply of
nutrients to the other tissues of the organismal soma,
sometimes at great 'tissue-personal' metabolic discom-
fiture. This phenomenon, which is named visceral-
gonadal transaction (VGT) is perceptible in several
organisms in sustaining the annual reproductive cycles
(Giese, 1959). Similar hepatic-heterohepatic (H-HH)
nutrient transactions may not be ruled out in the pre-

sent organism under the duress of the stressants (Epilogram II: Fig. VII 9.6). The occurrence of stressant-induced H-HH transactions suggested, is only a tentative suggestion based on circumstantial evidence and requires

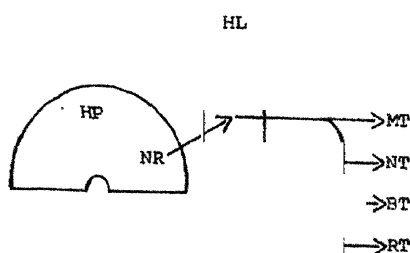


FIG. VII 9.6: EPILOGOGRAM II: THE NUTRIENT RESERVES (NR) OF HEPATOPANCREAS (HP) MAY BE SPARED (AFTER ALLOWANCE IS MADE FOR THE HEPATOPANCREATIC ENDOGENOUS METABOLISM) FOR SUPPLY TO OTHER TISSUES LIKE MUSCULAR TISSUE (MT), NEURAL TISSUE (NT), BRANCHIAL TISSUE (BT) AND REPRODUCTIVE TISSUE (RT), THROUGH THE MEDIATION OF HAEMOLYPH (HL). THIS TRANSACTION BETWEEN HEPATIC TISSUE (H) AND HETERO-HEPATIC (OTHER THAN HEPATIC) TISSUES (HH) MAY BE 'NORMALLY' OPERATIONAL IN THE UNSTRESSED ORGANISM. AND THIS H-HH TRANSACTION MAY BE EMPHASIZED UNDER THE STRESSANT DURESS SO AS TO CAUSE A PERCEPTIBLE REDUCTION IN THE GRAVIMETRIC STATUS OF HEPATOPANCREATIC TISSUE ITSELF.

closer experimental scrutiny before being accepted as a phenomenology connected with stressant intoxication.

VII 9.G ROLE OF CTGM

The via-hormonal influence of the central nervous system (the cephalothoracic ganglionic mass, CTGM) on the carbohydrate composition of the tissues of O. senex senex under the different stressant regimes has been mentioned earlier (see Chapterule VII 4.D). The modification of pool sizes of both TAEAPS (total acid-extractable anthrone-

positive substances) and TAPAPS (total acid-precipitable anthrone-positive substances) of the TCHR (total carbohydrate reserves) component occurs under the hegemony of stressants. The presidency of stressants over the metabolic events of alteration of sizes of TAEAPS and TAPAPS in tissues needs be in no doubt. Involvement of nervous system via-hormonal mechanisms is only suggested here as a matter of course and it required experimental probation. However, in as much as it provides the conceptual basis for the stressant's work in the organism, it may be accepted as a working hypothesis.

The CTGM of crab has been found to undergo noteworthy changes in its biochemical composition and activity profiles of enzyme systems (Table VII 2.1). Such an altered compositional catalytic state of the tissue under the stressant-hegemony involve altered secretory characteristics too — in the direction of elaboration of tissue TAEAPS and TAPAPS pool size modulatory principle(s), for example: Such a secretory inferential-proposition will accord well with the hormonal hypothesis proposed above.

Even if the via-hormonal mechanism comes to be established as a working principle for the operation of the stressants in the intra-organismal milieu, there will be no gainsaying the fact that the stressants resort

to direct action on the biochemical tissue targets. Precisely how much is contributed by each of these two 'modi operandia' of the stressants in the 'toxicophany' (show of toxicity) belongs to future dates when appropriate datal bases become available.

One more biochemical datal-point of CTGM with physiological poignancy needs a comment here: It is the modulation of the acetylcholine esterase system (AChE) of the tissue by the stressants. The positive change, in general, effected by the stressant regimes in the activity of AChE may stand for increased excitability of the nervous system under the aegis of the stressants. What are the effects of this probable hyperexcitable state of the nervous system on the physiological statuses of tissue-targets like muscle? What is the energetic implication of this 'effect', if any, in the target organ? Is the energetic state alteration presumed to occur in tissues like muscle under the stressant aegis (see the Chapterule VII 7) contributed by this 'via-neural' influence of the stressants?

Is this stressogenous neural hyperexcitability shared by the heart which has neural governance over its pulsational rhythmicity?

These and several other interrogatives that may arise out of this hypothetical situation will provide new inquiry-directions in the field of physiological toxicology (Fig. VII 9.7).

VII 9.H ABSENCE OF
POTENTIATION

One may recall here the identification of phenomenon of potentiation in but a couple of instances amidst a maze of data mentioned elsewhere (Chapterule VII 6). This can be taken for practical purposes as the absence of potentiatory interaction between the stressants studied here. This absence of potentiation may provide a reassurance to the environmental biologist that the biological system may not be unduly strained under the combined presence of the stressants in emerging ecopollution situations.

But of what avail can this assurance be in the present organism especially in a situation where the physiological status of the central metabolic organ is greatly affected, if the change in its weight status under the stressant-hegemony is any indication.

This brings one to the point

VII 9.I STRESSOGENOUS of 'stressogenous' atrophy of
 ATROPHY OF
 HEPATOPANCREAS hepatopancreas: Decrease of
 AND.... weight status of hepatopancreas

under the stressant-aegis may be taken as atrophic modification of this organ.

Given the metabolic catholicity of this tissue, this decrease of hepatopancreatic weight and its biochemical quantitative implications may be involved in the H-HH transactions noted above (VII 9.F). This is only the secondary consideration. The primary consideration is that, this decrease of weight of the central metabolic organ is shared as a 'response' by several diverse stressants when they are in action on the 'stressee' organisms.

Does this atrophy of the central metabolic organ form a part of the primary response repertoire of the stressee in the stressant-stressee interaction ?

Coming to the secondary consideration mentioned above, H-HH transactions, what is the sustenance-value of this phenomenology in the context of stress-induced metabolic demands that the conservative tissues of the organism may face ?

A small exercise of balance-striking using the nutrient metaquantigrams (Fig. VII 8.4) may show (Fig. VII 9.6) that hepatopancreatic nutrient-sparing catholi-

city is quite insufficient to explain certain 'gains' noted in the 'recipient' tissues.

In the Cd 30 dps regime, the loss in hepatopancreatic TL (201 mg) cannot fully account for the gains in haemolymph (200 mg) and other tissues (55.5 mg) put together. Under pH 15 dps regime, the TP pool suffers from this inadequate accountability (Table VII 9.45).

TABLE VII 9.45: Balance-striking exercise with the nutrient metaquantigrams of Fig. VII 8.4.

Regime		Component	Loss in HP (mg)	Gain in HL (mg)	Gain in other tissues (M + G) (mg)
Cd	30 dps	TL	201	200	55.5
pH	15 dps	TP	586	-	1977
pH	30 dps	TL	196	229.	566
Comb	15 dps	TL	116	196	716.5
Comb	30 dps	TL	404	309	131.5

<hr/>					

The disparity between loss of hepatopancreatic protein (586 mg) and gain in hetero-hepatic tissue grouping (1977 mg) is a yawning quantum of 1391 mg!! Similar disparities are noted in the case of

lipid pool under other regimes. These disparities can be explained only when other sources are invited into the account. The nutrient pools of other tissues (for example skeletal tissue) may be considered in this connection. Biological commonsense point may not permit such consideration. It is doubtful if the other tissues line up on the side of catholicity with the hepatopancreas.

The most important nutrient source that is often not given its due consideration in such toxicophysiological studies, as the present one, is the 'alimentary input': Unless the dynamics of this input under stressful regime is put in proper perspective, one may not get full account of the nutrient quanti-dynamics in the organism under stress.

This being the inevitability of alimentary input in the nutrient quanti-dynamics, in the present organism, stressed only sublethally, the author has noticed a distinct tendency of feeding behaviour: Drift in the direction of rejection of food offered to the organism during the prandial sessions. This loss of appetite, the 'stressogenous anorexia' has been noted to be perceptible around fifteenth day post-stress and becomes conspicuous by the fourth week of stress. (It is of interest to mention here a similar salogenous

anorexia that the same crab experiences under salinity stress, Venkata Reddy, 1976). The implication of this process in the nutrient quanti-dynamics should at once be apparent: Gradual closure of the alimentary gate and stoppage of the nutrient supply through this gate.

Then, the survival of the 'apparently' healthy organism through prolonged periods of stress is doubtful what with the fluidization of chelate leg muscle noted in the stressed organism, which casts a serious doubt on the continued functional efficiency of this inexorably degenerating tissue.

All this means, that the expression 'sublethal concentration', formulated in the second chapteral location of this dissertation, needs be taken with a pinch of salt.

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RÉSUMÉ

1. In vivo individual (in severo) and combinational (in combinatio) influence of Cadmium (Cd) and pH on the organismal metabolism and tissue biochemistry were studied in the local crab, Oziotelphusa senex senex.
2. The rate of oxygen consumption (expressed as Unit metabolism) was generally depressed by both individual and combinational regimes of Cd and pH.
3. The tissue carbohydrates were generally depressed under the different stressant regimes.
4. The tissue protein pools were generally depressed under the different stressant regimes.
5. The tissue lipid pools were generally depressed.
6. The tissue pools of total ninhydrin-positive substances (TNPS) exhibited general elevatory trend.
7. Haemolymph lipid pool showed a consistent elevatory trend under the different stressant regimes. This important hyperlipaemic effect is suggested to play in haemo detoxificatory role in the organism under the stressant duress.
8. The activity level of acetylcholine esterase (AChE) has been found to be elevated under the different

stressant regimes in the chelate leg muscle (M) and the cephalothoracic ganglionic mass (CTGM) of the organism.

9. The activity levels of aminotransferases were found to be elevated in the hepatopancreas (HP), M and CTGM.
10. The activity levels of ATPases were generally elevated in the tissues of the crab under the stressant regimes.
11. The activity levels of dehydrogenases were generally elevated.
12. The hepatopancreas showed an important trend of depression of its gravimetric status (weight status) under the different regimes of stressants. This 'stressogenous atrophy' of hepatopancreas has implications in the definition of toxicity levels of the stressants.
13. The important computational outcome of the stressogenous atrophy of hepatopancreas and the 'stressogenous hypertrophy' of muscle (noted in the crab under the different stressant regimes) is the calculation of weight-specific levels of the biochemical constituents and biocatalytic potentialities into the total weight of the tissue — an exercise named

holohistometry (total tissue level measurement).
This forms additional elucidational dimension
to the effect of stressants on the biochemistry
and physiology of 'stressee'.

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